

Oral dosages according to the present invention will include from about 50 to about 100 times the active ingredient, e.g., from about 500 to about 12,000 μg per day in single or divided doses, preferably from about 500 to about 5,000 μg per day. Pulmonary dosages according to the present invention will include from about 10 to about 100 times the active ingredient, e.g., from about 100 to about 12,000 μg per day in single or divided doses, preferably about 500 to 1000 μg per day. Nasal, buccal and sublingual dosages according to the present invention will also include from about 10 to about 100 times the active ingredient, e.g., from about 100 to about 12,000 μg per day in single or divided doses.

NOVEL EXENDIN AGONIST FORMULATIONS AND
METHODS OF ADMINISTRATION THEREOF

RELATED APPLICATIONS

- 5 *Sub* C This application claims priority from U.S. Provisional Application 60/116,380, entitled "Novel Exendin Agonist Formulations And Methods Of Administration Thereof," filed January 14, 1999, and U.S. Provisional Application 60/[not yet assigned], entitled "Use of Exendins and Agonists
- 10 Thereof for Modulation of Triglyceride Levels and Treatment of Dyslipidemia," filed January 14, 1999, the contents of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

- 15 The present invention relates to novel exendin and peptide exendin agonist formulations, dosages, and dosage formulations that are bioactive and are deliverable via injectable and non-injectable routes, for example, via the
- 20 respiratory tract, the mouth, and the gut. These formulations and dosages and methods of administration are useful in the treatment of diabetes, including Type I and II diabetes, in the treatment of disorders which would be benefited by agents which lower plasma glucose levels, and
- 25 in the treatment of disorders which would be benefited by the administration of agents useful in delaying and/or slowing gastric emptying or reducing food intake.

BACKGROUND

- 30 The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is

prior art to the presently claimed inventions, or relevant, nor that any of the publications specifically or implicitly referenced are prior art.

The extendins are peptides that are found in the
5 salivary secretions of the Gila monster and the Mexican Beaded Lizard, reptiles that are indigenous to Arizona and Northern Mexico. Exendin-3 [SEQ. ID. NO. 1: His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly
10 Ala Pro Pro Pro Ser-NH₂] is present in the salivary secretions of *Heloderma horridum* (Mexican Beaded Lizard), and exendin-4 [SEQ. ID. NO. 2: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro
15 Pro Ser-NH₂] is present in the salivary secretions of *Heloderma suspectum* (Gila monster) (Eng, J., et al., J. Biol. Chem., 265:20259-62, 1990; Eng, J., et al., J. Biol. Chem., 267:7402-05, 1992). The amino acid sequence of exendin-3 is shown in Figure 1. The amino acid sequence of exendin-4 is
20 shown in Figure 2. Exendin-4 was first thought to be a (potentially toxic) component of the venom. It now appears that exendin-4 is devoid of toxicity, and that it instead is made in salivary glands in the Gila monster.

The extendins have some sequence similarity to several
25 members of the glucagon-like peptide family, with the highest homology, 53%, being to GLP-1[7-36]NH₂ [SEQ. ID. NO. 3] (Goke, et al., J. Biol. Chem., 268:19650-55, 1993). GLP-1[7-36]NH₂ is also known as proglucagon[78-107], or simply "GLP-1" as used most often herein. GLP-1 has an
30 insulinotropic effect, stimulating insulin secretion from

3.

pancreatic beta cells. GLP-1 has also been reported to inhibit glucagon secretion from pancreatic alpha-cells (Ørsov, et al., Diabetes, 42:658-61, 1993; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). The amino acid sequence of GLP-1 is shown in Figure 3. GLP-1 has been reported to inhibit gastric emptying (Willms B, et al., J Clin Endocrinol Metab 81 (1): 327-32, 1996; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993), and gastric acid secretion (Schjoldager BT, et al., Dig Dis Sci 34 (5): 703-8, 1989; O'Halloran DJ, et al., J Endocrinol 126 (1): 169-73, 1990; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993)). GLP-1[7-37], which has an additional glycine residue at its carboxy terminus, also stimulates insulin secretion in humans (Ørsov, et al., Diabetes, 42:658-61, 1993). A transmembrane G-protein adenylate-cyclase-coupled receptor said to be responsible at least in part for the insulinotropic effect of GLP-1 has reportedly been cloned from a beta-cell line (Thorens, Proc. Natl. Acad. Sci. USA 89:8641-45, 1992).

GLP-1 has been the focus of significant investigation in recent years due to reported actions such as the amplification of stimulated insulin production (Byrne MM, Goke B. Lessons from human studies with glucagon-like peptide-1: Potential of the gut hormone for clinical use. In: Fehmman HC, Goke B. Insulinotropic Gut Hormone Glucagon-Like Peptide 1. Basel, Switzerland: Karger, 1997:219-33), the inhibition of gastric emptying (Wettergren A, et al., Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man, Dig. Dis. Sci. 1993 Apr;38(4):665-73), the inhibition of glucagon

secretion (Creutzfeldt WOC, et al., Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in type I diabetic patients, Diabetes Care 1996;19(6):580-6), and a purported role in appetite control (Turton MD, et al., A role for glucagon-like peptide-1 in the central regulation of feeding, Nature 1996 Jan;379(6560):69-72). GLP-1 has also been reported to restore islet glucose sensitivity in aging rats, restoring their glucose tolerance to that of younger rats (Egan JM, et al., Glucagon-like peptide-1 restores acute-phase insulin release to aged rats, Diabetologia 1997 June 40(Suppl 1):A130). The short duration of biological action of GLP-1 in vivo is one feature of the peptide that has hampered its development as a therapeutic agent.

Pharmacological studies have demonstrated both similarities and differences between exendin-4 and GLP-1. Exendin-4 reportedly can act at GLP-1 receptors on insulin-secreting β TC1 cells, at dispersed acinar cells from guinea pig pancreas, and at parietal cells from stomach. The peptide is also reported to stimulate somatostatin release and inhibit gastrin release in isolated stomachs (Goke, et al., J. Biol. Chem. 268:19650-55, 1993; Schepp, et al., Eur. J. Pharmacol., 69:183-91, 1994; Bissele, et al., Life Sci., 55:629-34, 1994). Exendin-3 and exendin-4 were reportedly found to stimulate cAMP production in, and amylase release from, pancreatic acinar cells (Malhotra, R., et al., Regulatory Peptides, 41:149-56, 1992; Raufman, et al., J. Biol. Chem. 267:21432-37, 1992; Singh, et al., Regul. Pept. 53:47-59, 1994). Exendin-4 also has a significantly longer duration of action than GLP-1. For example, in one

experiment, glucose lowering by exendin-4 in diabetic mice was reported to persist for several hours, and, depending on dose, for up to 24 hours (Eng J. Prolonged effect of exendin-4 on hyperglycemia of db/db mice, Diabetes 1996 May; 45(Suppl 2):152A (abstract 554)). Based on their
5 insulinotropic activities, the use of exendin-3 and exendin-4 for the treatment of diabetes mellitus and the prevention of hyperglycemia has been proposed (Eng, U.S. Patent No. 5,424,286).

10 C-terminally truncated exendin peptides such as exendin-4[9-39], a carboxyamidated molecule, and fragments 3-39 through 9-39 have been reported to be potent and selective antagonists of GLP-1 (Goke, et al., J. Biol. Chem., 268:19650-55, 1993; Raufman, J.P., et al., J. Biol. Chem. 266:2897-902, 1991; Schepp, W., et al., Eur. J. Pharm. 269:183-91, 1994; Montrose-Rafizadeh, et al., Diabetes, 45(Suppl. 2):152A, 1996). Exendin-4[9-39] is said to block endogenous GLP-1 in vivo, resulting in reduced insulin secretion. Wang, et al., J. Clin. Invest., 95:417-21, 1995;
15 D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). A receptor apparently responsible for the insulinotropic effect of GLP-1 in rats has reportedly been cloned from rat pancreatic islet cell (Thorens, B., Proc. Natl. Acad. Sci. USA 89:8641-8645, 1992). Exendins and exendin-4[9-39] are
20 said to bind to the cloned rat GLP-1 receptor (rat pancreatic β -cell GLP-1 receptor (Fehmann HC, et al., Peptides 15 (3): 453-6, 1994) and human GLP-1 receptor (Thorens B, et al., Diabetes 42 (11): 1678-82, 1993)). In cells transfected with the cloned GLP-1 receptor, exendin-4
30 is reportedly an agonist, i.e., it increases cAMP, while

exendin[9-39] is identified as an antagonist, i.e., it blocks the stimulatory actions of exendin-4 and GLP-1. Id.

Exendin-4[9-39] is also reported to act as an antagonist of the full length exendins, inhibiting stimulation of pancreatic acinar cells by exendin-3 and exendin-4 (Raufman, et al., J. Biol. Chem. 266:2897-902, 1991; Raufman, et al., J. Biol. Chem., 266:21432-37, 1992). It is also reported that exendin[9-39] inhibits the stimulation of plasma insulin levels by exendin-4, and inhibits the somatostatin release-stimulating and gastrin release-inhibiting activities of exendin-4 and GLP-1 (Kolligs, F., et al., Diabetes, 44:16-19, 1995; Eissele, et al., Life Sciences, 55:629-34, 1994). Exendin [9-39] has been used to investigate the physiological relevance of central GLP-1 in control of food intake (Turton, M.D. et al. Nature 379:69-72, 1996). GLP-1 administered by intracerebroventricular injection inhibits food intake in rats. This satiety-inducing effect of GLP-1 delivered ICV is reported to be inhibited by ICV injection of exendin [9-39] (Turton, supra). However, it has been reported that GLP-1 does not inhibit food intake in mice when administered by peripheral injection (Turton, M.D., Nature 379:69-72, 1996; Bhavsar, S.P., Soc. Neurosci. Abstr. 21:460 (188.8), 1995).

The results of an investigation of whether exendins are the species homolog of mammalian GLP-1 was reported by Chen and Drucker who cloned the exendin gene from the Gila monster (J. Biol. Chem. 272(7):4108-15 (1997)). The observation that the Gila monster also has separate genes for proglucagons (from which GLP-1 is processed), that are

more similar to mammalian proglucagon than exendin, indicates that exendins are not species homologs of GLP-1.

Agents that serve to delay gastric emptying have found a place in medicine as diagnostic aids in gastrointestinal radiological examinations. For example, glucagon is a polypeptide hormone that is produced by the alpha cells of the pancreatic islets of Langerhans. It is a hyperglycemic agent that mobilizes glucose by activating hepatic glycogenolysis. It can to a lesser extent stimulate the secretion of pancreatic insulin. Glucagon is used in the treatment of insulin-induced hypoglycemia, for example, when administration of glucose intravenously is not possible. However, as glucagon reduces the motility of the gastrointestinal tract it is also used as a diagnostic aid in gastrointestinal radiological examinations. Glucagon has also been used in several studies to treat various painful gastrointestinal disorders associated with spasm. Daniel, et al. (Br. Med. J., 3:720, 1974) reported quicker symptomatic relief of acute diverticulitis in patients treated with glucagon compared with those who had been treated with analgesics or antispasmodics. A review by Glauser, et al. (J. Am. Coll. Emergency Physng, 8:228, 1979) described relief of acute esophageal food obstruction following glucagon therapy. In another study, glucagon significantly relieved pain and tenderness in 21 patients with biliary tract disease compared with 22 patients treated with placebo (M.J. Stower, et al., Br. J. Surg., 69:591-2, 1982).

Methods for regulating gastrointestinal motility using amylin agonists are described in commonly owned

International Application No. PCT/US94/10225, published March 16, 1995.

Methods for regulating gastrointestinal motility using exendin agonists are described in commonly owned U.S. Patent Application Serial No. 08/908,867, filed August 8, 1997
5 entitled "Methods for Regulating Gastrointestinal Motility," which application is a continuation-in-part of U.S. Patent Application Serial No. 08/694,954 filed August 8, 1996.

Methods for reducing food intake using exendin agonists
10 are described in commonly owned U.S. Patent Application Serial No. 09/003,869, filed January 7, 1998, entitled "Use of Exendin and Agonists Thereof for the Reduction of Food Intake," which claims the benefit of U.S. Provisional Application Nos. 60/034,905 filed January 7, 1997,
15 60/055,404 filed August 7, 1997, 60/065,442 filed November 14, 1997 and 60/066,029 filed November 14, 1997.

Exendins have also been reported to have inotropic and diuretic effects, as set forth in commonly owned International Application No. PCT/US99/02554, filed February
20 5, 1999, claiming the benefit of Provisional Application No. 60/075,122, filed February 13, 1998.

Novel exendin agonist compounds are described in commonly owned PCT Application Serial No. PCT/US98/16387 filed August 6, 1998, entitled "Novel Exendin Agonist
25 Compounds," which claims the benefit of U.S. Patent Application Serial No. 60/055,404, filed August 8, 1997.

Other novel exendin agonists are described in commonly owned PCT Application Serial No. PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist

Compounds," which claims the benefit of U.S. Provisional Application No. 60/065,442 filed November 14, 1997.

Still other novel extendin agonists are described in commonly owned PCT Application Serial No. PCT/US98/24273, filed November 13, 1998, entitled "Novel Extendin Agonist Compounds," which claims the benefit of U.S. Provisional Application No. 60/066,029 filed November 14, 1997.

Since the appearance of the first therapeutically active peptides and proteins produced by genetic engineering, there has been an ever-increasing demand to be able to deliver these drugs by routes other than parenteral. This has been thwarted, however, by the very properties of peptides and proteins that set them apart from the small drug molecules widely in use today. These properties include molecular size, susceptibility to proteolytic breakdown, rapid plasma clearance, peculiar dose-response curves, immunogenicity, biocompatibility, and the tendency of peptides and proteins to undergo aggregation, adsorption, and denaturation.

It is generally understood that the administration of peptide drugs by routes other than subcutaneous or intravenous injection, or intravenous infusion, is often not practical because of, for example, in the case of oral administration, both enzymatic degradation and non-absorption in the gastrointestinal tract. Thus, there continues to exist a need for the development of alternative methods to the inconvenient, sometimes painful, injection for administration of peptide drugs, such as extendins and the peptide extendin agonist analogs referenced above. In addition to formulations and dosages useful in the

administration of exendins and exendin agonists by injection, formulations, dosage formulations, and methods that solve these problems and that are useful in the non-injection delivery of therapeutically effective amounts of exendin and exendin agonists are described and claimed herein.

The contents of the above-identified articles, patents, and patent applications, and all other documents mentioned or cited herein, are hereby incorporated by reference in their entirety. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other documents mentioned or cited herein.

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides novel exendin and exendin agonist compound formulations and dosages thereof exhibiting advantageous properties that include effects in slowing gastric emptying and lowering plasma glucose levels. Thus, this aspect of the invention includes formulations of exendins and exendin agonists that comprise an exendin or exendin agonist mixed together with a buffer (preferably an acetate buffer), an iso-osmolality modifier (preferably mannitol), and optionally containing a preservative (preferably m-cresol), said formulation having a pH of between about 3.0 and about 7.0 (preferably between about 4.0 and about 5.0). By an "exendin agonist" is meant a compound that mimics one or more effects of exendin, for example, by binding to a receptor where exendin causes one or more of these effects, or by activating a signaling

cascade by which exendin causes one or more of these effects. Exendin agonists include exendin agonist peptides, such as analogs and derivatives of exendin-3 and exendin-4 that have one or more desired activities of exendin.

- 5 Various exendin agonist analogs are identified or referenced herein.

Additional exendin and exendin agonist formulations within the scope of the invention include a parenteral liquid dosage form, a lyophilized unit-dosage form, a
10 lyophilized multi-use dosage form, and modifications of these dosage forms that are useful in the oral, nasal, buccal, sublingual, intra-tracheal, and pulmonary delivery of exendins and exendin agonists.

- Thus, the invention includes parenteral liquid dosage
15 forms that comprise approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or from about 0.005 to about 0.05% (w/v), respectively of the active ingredient in an aqueous system along with approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or
20 glutamate or similar buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0, as well as either approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol iso-
25 osmolality modifier (preferably mannitol) or up to about 0.9% saline or a combination of both leading to an isotonic or an iso-osmolar solution in an aqueous continuous phase. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the group consisting of m-cresol,
30 benzyl alcohol, methyl, ethyl, propyl and butyl parabens and

phenol is also present if the formulation is packaged in a multi-use container. A sufficient amount of water for injection is added to obtain the desired concentration of solution. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, must maintain the overall stability of the active ingredient. Useful polyhydric alcohols include such compounds as sorbitol, mannitol, glycerol, and polyethylene glycols (PEGs). The polyhydric alcohols and the carbohydrates will also be effective in stabilizing protein against denaturation caused by elevated temperature and by freeze-thaw or freeze-drying processes. Suitable carbohydrates include galactose, arabinose, lactose or any other carbohydrate which does not have an adverse affect on a diabetic patient, if intended for that use, i.e., the carbohydrate is not metabolized to form large concentrations of glucose in the blood. Preferably, the peptides of the present invention are admixed with a polyhydric alcohol such as sorbitol, mannitol, inositol, glycerol, xylitol, and polypropylene/ethylene glycol copolymer, as well as various polyethylene glycols (PEG) of molecular weight 200, 400, 1450, 3350, 4000, 6000, and 8000). Mannitol is the preferred polyhydric alcohol.

The lyophilized unit-dose formulations of the present invention are also stable, but need not be isotonic and/or iso-osmolar. They include active ingredient(s), a bulking agent to facilitate cake formation (which may also act as a tonicifer and/or iso-osmolality modifier upon reconstitution to either facilitate stability of the active ingredient and/or lessen the pain on injection), and may also include a

surfactant that benefits the properties of the cake and/or facilitates reconstitution. The lyophilized unit-dose formulations of the present invention include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or 0.005 to 0.05% (w/v) of the active ingredient. It may not be necessary to include a buffer in the formulation and/or to reconstitute the lyophile with a buffer if the intention is to consume the contents of the container within the stability period established for the reconstituted active ingredient. If a buffer is used, it may be included in the lyophile or in the reconstitution solvent. Therefore, the formulation and/or the reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol iso-osmolality modifier (as described above) or up to 0.9% saline or a combination of both leading to a isotonic or iso-osmolar solution in the reconstituted aqueous phase. A surfactant, preferably about 0.1 to about 1.0% (w/v) of polysorbate 80 or other non-ionic detergent, may be included. As noted above, sodium chloride, as well as other excipients, may also be present in the lyophilized unit-dosage formulation, if desired. The liquid formulation of the invention prior to lyophilization will be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation

of isotonic and/or iso-osmolar solutions after reconstitution.

The invention also includes lyophilized and liquid multi-dose formulations. As with the parenteral liquid and lyophilized unit-dosage formulations described above, the lyophilized multi-unit-dosage form should contain a bulking agent to facilitate cake formation. A preservative is included to facilitate multiple use by the patient. These dosage forms include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or from about 0.005 to 0.05% (w/v), respectively of the active ingredient. If a buffer is used, it may be included in the lyophile or in the reconstitution solvent, and the formulation and/or the reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either approximately 1.0 to 10% (w/v) of a carbohydrate or a polyhydric alcohol iso-osmolality modifier (preferably mannitol) or up to 0.9% saline, or a combination of both, leading to an isotonic or iso-osmolar solution in the reconstituted aqueous phase. A surfactant, preferably about 0.1 to about 1.0% (w/v) of polysorbate 80 or other non-ionic detergent, may be included. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol (preferably m-cresol) is also present if the

formulation is packaged in a multi-use container. Sodium chloride, as well as other excipients, may also be present, if desired. The liquid formulation of the invention should be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation of isotonic and/or iso-osmolar solutions after reconstitution.

The invention further includes solid dosage forms useful for oral, buccal, sublingual, intra-tracheal, nasal, and pulmonary delivery. The formulations that best support pulmonary and/or intra-tracheal dosage forms may be either preserved or unpreserved liquid formulations and/or dry powder formulations. The preserved or unpreserved liquid formulations will be essentially identical to the formulations described above under preserved or unpreserved liquid parenteral formulations. The pH of the solution should be about 3.0 to 7.0, more specifically from about 4.0 to 6.0, or from about 4.0 to 5.0, with a pH greater than or equal to about 5.0 being most preferred to reduce the potential for bronchoconstriction. The dry powder formulations may contain a bulking agent and/or salts to facilitate particle size formation and appropriate particle size distribution. A surfactant and/or salts may also benefit the properties of the particle morphology and/or facilitate tissue uptake of the active ingredient. Dry powder dosage forms can range from 1% to 100% (w/w), respectively of the active ingredient. It may not be necessary to include a bulking agent and/or salts to facilitate particle size formation and/or distribution. The bulking agent and/or salts may consist of either approximately 0 to 99% (w/w) of a carbohydrate or polyhydric

alcohol or approximately 0 to 99% salt or a combination of both leading to the preferred particle size and distribution. A surfactant, preferably about 0.1 to about 1.0% (w/w) of polysorbate 80 or other non-ionic detergent, may be included. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, will maintain the overall stability of the active ingredient and facilitate the proper level of hydration.

Also within the scope of the invention is the formulation comprising up to 50 mg/ml of an exendin or an exendin agonist in 30mM acetate buffer (pH about 4.5) and mannitol, with or without a preservative.

Further within the scope of the invention are preferred dosages for exendins and exendin agonists when given by injection, and when given by other routes. Thus, formulations for exendin and exendin agonists having comparable potency are provided for the administration by injection of from about 0.1 to about 0.5 µg per kilogram, given one to three times per day. Typically, for the patient with diabetes who weighs in the range from about 70 kilograms (average for the type 1 diabetic) to about 90 kilograms (average for the type 2 diabetic), for example, this will result in the total administration of about 10 to about 120 µg per day in single or divided doses. If administered in divided doses, the doses are preferably administered two or three times per day, and more preferably, two times per day.

In a preferred injection procedure, the exendin or exendin agonist is administered parenterally, more preferably

by injection, for example, by peripheral injection. Preferably, about 1 μg -30 μg to about 1 mg of the exendin or exendin agonist is administered per day. More preferably, about 1-30 μg to about 500 μg , or about 1-30 μg to about 50

5 μg of the exendin or exendin agonist is administered per day. Most preferably, depending upon the weight of the subject and the potency of the compound administered, about 3 μg to about 50 μg of the exendin or exendin agonist is administered per day. Preferred doses based upon patient weight for compounds

10 having approximately the potency of exendin-4 range from about 0.005 $\mu\text{g}/\text{kg}$ per dose to about 0.2 $\mu\text{g}/\text{kg}$ per dose. More preferably, doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.02 $\mu\text{g}/\text{kg}$ per dose to about 0.1 $\mu\text{g}/\text{kg}$ per dose. Most

15 preferably, doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.05 $\mu\text{g}/\text{kg}$ per dose to about 0.1 $\mu\text{g}/\text{kg}$ per dose. These doses are administered from 1 to 4 times per day, preferably from 1 to 2 times per day. Doses of exendins or exendin

20 agonists will normally be lower if given by continuous infusion. Doses of exendins or exendin agonists will normally be higher if given by non-injection methods, such as oral, buccal, sublingual, nasal, pulmonary or skin patch delivery.

25 Oral dosages according to the present invention will include from about 50 to about 100 times the active ingredient, ^{e.g.} ~~i.e.~~, from about 500 to about 12,000 μg per day in single or divided doses, preferably from about 500 to about 5,000 μg per day. Pulmonary dosages according to the

present invention will include from about 10 to about 100 times the active ingredient, ~~i.e.~~^{e.g.}, from about 100 to about 12,000 µg per day in single or divided doses, preferably about 500 to 1000 µg per day. Nasal, buccal and sublingual dosages according to the present invention will also include from about 10 to about 100 times the active ingredient, ~~i.e.~~^{e.g.}, from about 100 to about 12,000 µg per day in single or divided doses.

Preferred dosages for nasal administration are from about 10-1000 to about 1200-12,000 µg per day, for buccal administration from about 10-1000 to about 1200-12,000 µg per day, and for sublingual administration from about 10-1000 to about 1200-8,000 µg per day. Sublingual dosages are preferably smaller than buccal dosages. Administration dosages for exendin agonists having less than or greater than the potency of exendin-4 are increased or decreased as appropriate from those described above and elsewhere herein.

Also included within the scope of the present invention are methods of administration of said novel exendin agonist compound formulations and dosages by delivery means alternative to subcutaneous injection or intravenous infusion, including, for example, by nasal delivery, pulmonary delivery, oral delivery, intra-tracheal delivery, sublingual delivery, and buccal delivery.

According to another aspect, the present invention provides novel exendin agonist compound formulations and dosages, and methods for the administration thereof, that are useful in treating diabetes (including type 1 and type 2 diabetes), obesity, and other conditions that will benefit

from the administration of a therapy that can slow gastric emptying, lowering plasma glucose levels, and reduce food intake.

The invention also includes methods for treatment of subjects in order to increase insulin sensitivity by administering an extendin or an extendin agonist. The extendin and extendin agonist formulations and dosages described herein may be used to increase the sensitivity of a subject to endogenous or exogenous insulin.

In one preferred aspect, the extendin or extendin agonist used in the methods of the present invention is extendin-3 [SEQ. ID. NO. 1]. In another preferred aspect, said extendin is extendin-4 [SEQ. ID. NO. 2]. Other preferred extendin agonists include extendin-4 (1-30) [SEQ ID NO 6: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly], extendin-4 (1-30) amide [SEQ ID NO 7: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-NH₂], extendin-4 (1-28) amide [SEQ ID NO 40: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂], ¹⁴Leu, ²⁵Phe extendin-4 [SEQ ID NO 9: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂], ¹⁴Leu, ²⁵Phe extendin-4 (1-28) amide [SEQ ID NO 41: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂], and ¹⁴Leu, ²²Ala, ²⁵Phe extendin-4 (1-28) amide [SEQ ID NO 8: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Ala Ile Glu Phe Leu Lys Asn-NH₂].

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

In accordance with the present invention and as used herein, the following terms are defined to have the following meanings, unless explicitly stated otherwise. "Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the amino acid sequence for exendin-3 [SEQ. ID. NO. 1].

Figure 2 depicts the amino acid sequence for exendin-4 [SEQ. ID. NO. 2].

Figure 3 depicts the amino acid sequence for GLP-1[7-36]NH₂ (GLP-1) [SEQ. ID. NO. 3].

Figure 4 depicts the plasma levels of exendin-4 in rats after intra-tracheal administration.

Figure 5a depicts the plasma exendin-4 concentration after intra-tracheal instillation in db/db mice.

Figure 5b depicts the effect of intra-tracheal administration of exendin-4 on plasma glucose in db/db mice.

Figures 6a and 6b depict the effect of intra-tracheal administration of exendin-4 on plasma glucose in ob/ob mice.

Figure 7a depicts the plasma exendin-4 concentration after intra-tracheal instillation into rats.

Figure 7b depicts the bioavailability of exendin-4 following intra-tracheal instillation into rats.

5 Figure 8 depicts plasma exendin-4 concentrations in rats exposed to aerosolized exendin-4.

Figure 9a depicts the effect of ten minutes of exposure to aerosolized exendin-4 on plasma glucose in db/db mice.

10 Figure 9b depicts the plasma exendin-4 concentration after ten minutes of exposure of db/db mice to aerosolized exendin-4.

Figure 10 depicts plasma exendin-4 concentrations in rats after intra-nasal administration of exendin-4.

15 Figure 11 depicts the effect of intra-gastric administration of exendin-4 on plasma glucose in db/db mice.

Figure 12a depicts the plasma exendin-4 concentration after sublingual administration to db/db mice.

Figure 12b depicts the effect of sublingual administration of exendin-4 on plasma glucose in db/db mice.

20 Figure 12c depicts the plasma exendin-4 concentration after sublingual administration to rats.

Figure 12d depicts the bioavailability of exendin-4 after sublingual administration.

Figure 12e depicts the Cmax of sublingual exendin-4.

25 Figure 13 depicts the effect of exendin-4 (administered i.p. twice daily) on food intake (a), change in body weight (b) (initial body weight $441 \pm 39\text{g}$), or change in hemoglobin A_{1c} (c) ($7.68 \pm 0.20\%$ at 0 weeks). Dose-responses (right panels) are for the means over the last 2 of 6 weeks
30 treatment.

Figure 14 depicts the plasma glucose concentration (a), glucose infusion rate required to maintain euglycemia (b) and plasma lactate concentration (c) in clamp procedures performed on ZDF rats previously treated for 6 weeks with the specified doses of exendin-4 (i.p. twice daily). Dose-responses for glucose infusion rate and plasma lactate concentration are based upon mean values obtained between 60 and 180 min of the clamp procedure.

Figure 15 depicts the amino acid sequences for certain exendin agonist compounds useful in the present invention [SEQ ID NOS 9-39].

Figures 16 and 17 depict glucose-lowering results from the clinical study described in Example 12.

DETAILED DESCRIPTION OF THE INVENTION

Exendins and Exendin Agonists

Exendin-3 and Exendin-4 are naturally occurring peptides isolated from the salivary secretions of the Gila monster and the Mexican Beaded Lizard. Animal testing of exendin-4 has shown that its ability to lower blood glucose persists for several hours. Exendin-4, a 39-amino acid polypeptide, is synthesized using solid phase synthesis as described herein, and this synthetic material has been shown to be identical to that of native exendin-4.

Various aspects of the nonclinical pharmacology of exendin-4 have been studied. In the brain, exendin-4 binds principally to the *area postrema* and *nucleus tractus solitarius* region in the hindbrain and to the subfornical organ in the forebrain. Exendin-4 binding has been observed

in the rat and mouse brain and kidney. The structures to which exendin-4 binds in the kidney are unknown.

A number of other experiments have compared the biologic actions of exendin-4 and GLP-1 and demonstrated a more favorable spectrum of properties for exendin-4. A single subcutaneous dose of exendin-4 lowered plasma glucose in *db/db* (diabetic) and *ob/ob* (diabetic obese) mice by up to 40%. In Diabetic Fatty Zucker (ZDF) rats, 5 weeks of treatment with exendin-4 lowered HbA_{1c} (a measure of glycosylated hemoglobin used to evaluate plasma glucose levels) by up to 41%. Insulin sensitivity was also improved by 76% following 5 weeks of treatment in obese ZDF rats. In glucose intolerant primates, dose-dependent decreases in plasma glucose were also observed. See also Example 6, which describes the results of an experiment indicating that exendin is more potent and/or effective than GLP-1 in amplifying glucose-stimulated insulin release. Example 8, furthermore, describes work showing that the ability of exendin-4 to lower glucose in vivo was 3430 times more potent than that of GLP-1.

An insulinitropic action of exendin-4 has also been observed in rodents, improving insulin response to glucose by over 100% in non-fasted Harlan Sprague Dawley (HSD) rats, and by up to ~10-fold in non-fasted *db/db* mice. Higher pretreatment plasma glucose concentrations were associated with greater glucose-lowering effects. Thus the observed glucose lowering effect of exendin-4 appears to be glucose-dependent, and minimal if animals are already euglycemic. Exendin-4 treatment is also associated with improvement in

glycemic indices and in insulin sensitivity, as described in Examples 9 and 13.

Exendin-4 dose dependently slowed gastric emptying in HSD rats and was -90-fold more potent than GLP-1 for this action. Exendin-4 has also been shown to reduce food intake in NIH/Sw (Swiss) mice following peripheral administration, and was at least 1000 times more potent than GLP-1 for this action. Exendin-4 reduced plasma glucagon concentrations by approximately 40% in anesthetized ZDF rats during hyperinsulinemic, hyperglycemic clamp conditions, but did not affect plasma glucagon concentrations during euglycemic conditions in normal rats. See Example 4. Exendin-4 has been shown to dose-dependently reduce body weight in obese ZDF rats, while in lean ZDF rats, the observed decrease in body weight appears to be transient.

Through effects on augmenting and restoring insulin secretion, as well as inhibiting glucagon secretion, exendin-4 will be useful in people with type 2 diabetes who retain the ability to secrete insulin. Its effects on food intake, gastric emptying, other mechanisms that modulate nutrient absorption, and glucagon secretion also support the utility of exendin-4 in the treatment of, for example, obesity, type 1 diabetes, and people with type 2 diabetes who have reduced insulin secretion.

The toxicology of exendin-4 has been investigated in single-dose studies in mice, rats, and monkeys, repeated-dose (up to 28 consecutive daily doses) studies in rats and monkeys and *in vitro* tests for mutagenicity and chromosomal alterations. To date, no deaths have occurred, and there have been no observed treatment-related changes in

hematology, clinical chemistry, or gross or microscopic tissue changes. Exendin-4 was demonstrated to be non-mutagenic, and did not cause chromosomal aberrations at the concentrations tested (up to 5000 µg/mL).

- 5 In support of the investigation of the nonclinical pharmacokinetics and metabolism of exendin-4, a number of immunoassays have been developed. A radioimmunoassay with limited sensitivity (~100 pM) was used in initial pharmacokinetic studies. A two-site IRMA assay for exendin-4 was subsequently validated with a lower limit of quantitation of 15 pM. See Examples 5 and 7. The bioavailability of exendin-4, given subcutaneously, was found to be approximately 50-80% using the radioimmunoassay. This was similar to that seen following intraperitoneal administration (48-60%). Peak plasma concentrations (C_{max}) occurred between 30 and 43 minutes (T_{max}). Both C_{max} and AUC values were monotonically related to dose. The apparent terminal half-life for exendin-4 given subcutaneously was approximately 90-110 minutes. This was significantly longer than the 14-41 minutes seen following intravenous dosing. Similar results were obtained using the IRMA assay. Degradation studies with exendin-4 compared to GLP-1 indicate that exendin-4 is relatively resistant to degradation.
- 15 Investigation of the structure activity relationship (SAR) to evaluate structures that may relate to the antidiabetic activity of exendin, for its stability to metabolism, and for improvement of its physical characteristics, especially as it pertains to peptide stability and to amenability to alternative delivery

systems, has led to the discovery of exendin agonist peptide compounds. Exendin agonists include exendin peptide analogs in which one or more naturally occurring amino acids are eliminated or replaced with another amino acid(s).

- 5 Preferred exendin agonists are agonist analogs of exendin-4. Particularly preferred exendin agonists those described in International Application No. PCT/US98/16387, filed August 6, 1998, entitled, "Novel Exendin Agonist Compounds," which claims the benefit of United States Provisional Application
- 10 No. 60/055,404, filed August 8, 1997, including compounds of the formula (I) [SEQ ID NO. 3]:

Xaa₁ Xaa₂ Xaa₃ Gly Thr Xaa₄ Xaa₅ Xaa₆ Xaa₇ Xaa₈

Ser Lys Gln Xaa₉ Glu Glu Glu Ala Val Arg Leu

- 15 Xaa₁₀ Xaa₁₁ Xaa₁₂ Xaa₁₃ Leu Lys Asn Gly Gly Xaa₁₄

Ser Ser Gly Ala Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈-Z

- wherein Xaa₁ is His, Arg or Tyr; Xaa₂ is Ser, Gly, Ala or Thr; Xaa₃ is Asp or Glu; Xaa₄ is Phe, Tyr or naphthylalanine;
- 20 Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu, Ile, Val, pentylglycine or Met; Xaa₉ is Leu, Ile, pentylglycine, Val or Met; Xaa₁₀ is Phe, Tyr or naphthylalanine; Xaa₁₁ is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp, Phe,
- 25 Tyr, or naphthylalanine; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa₁₈ is Ser, Thr or Tyr; and Z is -OH or -NH₂; with the proviso that the compound is not exendin-3 or exendin-4.

Preferred N-alkyl groups for N-alkylglycine, N-alkylpentylglycine and N-alkylalanine include lower alkyl groups preferably of 1 to about 6 carbon atoms, more preferably of 1 to 4 carbon atoms. Suitable compounds

5 include those listed in Figure 1 having amino acid sequences of SEQ. ID. NOS. 9 to 39.

Preferred exendin agonist compounds include those wherein Xaa₁ is His or Tyr. More preferably, Xaa₁ is His.

Preferred are those compounds wherein Xaa₂ is Gly.

10 Preferred are those compounds wherein Xaa₃ is Leu, pentylglycine, or Met.

Preferred compounds include those wherein Xaa₁₃ is Trp or Phe.

Also preferred are compounds where Xaa₄ is Phe or

15 naphthylalanine; Xaa₁₁ is Ile or Val and Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-alkylalanine. Preferably N-alkylalanine has a N-alkyl group of 1 to about 6 carbon atoms.

According to an especially preferred aspect, Xaa₁₅, Xaa₁₆

20 and Xaa₁₇ are the same amino acid residue.

Preferred are compounds wherein Xaa₁₈ is Ser or Tyr, more preferably Ser.

Preferably Z is -NH₂.

According to one aspect, preferred are compounds of

25 formula (I) wherein Xaa₁ is His or Tyr, more preferably His; Xaa₂ is Gly; Xaa₄ is Phe or naphthylalanine; Xaa₃ is Leu, pentylglycine or Met; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile or Val; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-

alkylalanine; and Xaa₁₈ is Ser or Tyr, more preferably Ser. More preferably Z is -NH₂.

According to an especially preferred aspect, especially preferred compounds include those of formula (I) wherein:

- 5 Xaa₁ is His or Arg; Xaa₂ is Gly; Xaa₃ is Asp or Glu; Xaa₄ is Phe or naphthylalanine; Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu or pentylglycine; Xaa₉ is Leu or pentylglycine; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile, Val or t-butyltylglycine; Xaa₁₂ is Glu or Asp;
- 10 Xaa₁₃ is Trp or Phe; Xaa₁₄, Xaa₁₅, Xaa₁₆, and Xaa₁₇ are independently Pro, homoproline, thioproline, or N-methylalanine; Xaa₁₈ is Ser or Tyr; and Z is -OH or -NH₂; with the proviso that the compound does not have the formula of either SEQ. ID. NOS. 1 or 2. More preferably, Z is -NH₂.
- 15 Especially preferred compounds include those having the amino acid sequence of SEQ. ID. NOS. 9, 10, 21, 22, 23, 26, 28, 34, 35 and 39.

- According to an especially preferred aspect, provided are compounds where Xaa₉ is Leu, Ile, Val or pentylglycine,
- 20 more preferably Leu or pentylglycine, and Xaa₁₃ is Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds will exhibit advantageous duration of action and be less subject to oxidative degradation, both in vitro and in vivo, as well as during synthesis of the compound.

- 25 Exendin agonist compounds also include those described in International Application No. PCT/US98/24210, filed November 13, 1998, entitled, "Novel Exendin Agonist compounds," which claims the benefit of United States Provisional Application No. 60/065,442, filed November 14,

1997, including compounds of the formula (II) [SEQ ID NO. 4]:

- Xaa₁ Xaa₂ Xaa₃ Gly Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
- 5 Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀
Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁; wherein
- Xaa₁ is His, Arg or Tyr;
Xaa₂ is Ser, Gly, Ala or Thr;
- 10 Xaa₃ is Asp or Glu;
Xaa₅ is Ala or Thr;
Xaa₆ is Ala, Phe, Tyr or naphthylalanine;
Xaa₇ is Thr or Ser;
Xaa₈ is Ala, Ser or Thr;
- 15 Xaa₉ is Asp or Glu;
Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;
Xaa₁₁ is Ala or Ser;
Xaa₁₂ is Ala or Lys;
Xaa₁₃ is Ala or Gln;
- 20 Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;
Xaa₁₅ is Ala or Glu;
Xaa₁₆ is Ala or Glu;
Xaa₁₇ is Ala or Glu;
Xaa₁₉ is Ala or Val;
- 25 Xaa₂₀ is Ala or Arg;
Xaa₂₁ is Ala or Leu;
Xaa₂₂ is Ala, Phe, Tyr or naphthylalanine;
Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine
or Met;
- 30 Xaa₂₄ is Ala, Glu or Asp;

Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;

Xaa₂₆ is Ala or Leu;

Xaa₂₇ is Ala or Lys;

Xaa₂₈ is Ala or Asn;

5 Z₁ is -OH,

-NH₂

Gly-Z₂,

Gly Gly-Z₂,

Gly Gly Xaa₃₁-Z₂,

10 Gly Gly Xaa₃₁ Ser-Z₂,

Gly Gly Xaa₃₁ Ser Ser-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,

15 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂ or

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂;

Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently Pro,

homoproline, 3Hyp, 4Hyp, thioproline,

N-alkylglycine, N-alkylpentylglycine or

20 N-alkylalanine; and

Z₂ is -OH or -NH₂;

provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈,

Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉,

Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala.

25 Preferred N-alkyl groups for N-alkylglycine, N-alkylpentylglycine and N-alkylalanine include lower alkyl groups preferably of 1 to about 6 carbon atoms, more preferably of 1 to 4 carbon atoms.

Preferred extendin agonist compounds include those

30 wherein Xaa₁ is His or Tyr. More preferably Xaa₁ is His.

Preferred are those compounds wherein Xaa₂ is Gly.

Preferred are those compounds wherein Xaa₁₄ is Leu, pentylglycine or Met.

Preferred compounds are those wherein Xaa₂₅ is Trp or

5 Phe.

Preferred compounds are those where Xaa₆ is Phe or naphthylalanine; Xaa₂₂ is Phe or naphthylalanine and Xaa₂₃ is Ile or Val.

Preferred are compounds wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and
10 Xaa₃₈ are independently selected from Pro, homoproline, thioproline and N-alkylalanine.

Preferably Z₁ is -NH₂.

Preferable Z₂ is -NH₂.

According to one aspect, preferred are compounds of
15 formula (II) wherein Xaa₁ is His or Tyr, more preferably His; Xaa₂ is Gly; Xaa₆ is Phe or naphthylalanine; Xaa₁₄ is Leu, pentylglycine or Met; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile or Val; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro, homoproline, thioproline or N-
20 alkylalanine. More preferably Z₁ is -NH₂.

According to an especially preferred aspect, especially preferred compounds include those of formula (II) wherein:
Xaa₁ is His or Arg; Xaa₂ is Gly or Ala; Xaa₃ is Asp or Glu; Xaa₅ is Ala or Thr; Xaa₆ is Ala, Phe or naphthylalanine; Xaa,
25 is Thr or Ser; Xaa₈ is Ala, Ser or Thr; Xaa₉ is Asp or Glu; Xaa₁₀ is Ala, Leu or pentylglycine; Xaa₁₁ is Ala or Ser; Xaa₁₂ is Ala or Lys; Xaa₁₃ is Ala or Gln; Xaa₁₄ is Ala, Leu or pentylglycine; Xaa₁₅ is Ala or Glu; Xaa₁₆ is Ala or Glu; Xaa₁₇ is Ala or Glu; Xaa₁₉ is Ala or Val; Xaa₂₀ is Ala or Arg; Xaa₂₁
30 is Ala or Leu; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile,

- Val or tert-butylglycine; Xaa₂₄ is Ala, Glu or Asp; Xaa₂₅ is Ala, Trp or Phe; Xaa₂₆ is Ala or Leu; Xaa₂₇ is Ala or Lys; Xaa₂₈ is Ala or Asn; Z₁ is -OH, -NH₂, Gly-Z₂, Gly Gly-Z₂, Gly Gly Xaa₃₁-Z₂, Gly Gly Xaa₃₁ Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇, Xaa₃₈-Z₂; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ being independently Pro homoproline, thioproline or N-methylalanine; and Z₂ being -OH or -NH₂; provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala. Especially preferred compounds include those having the amino acid sequence of SEQ. ID. NOS. 40-61.
- According to an especially preferred aspect, provided are compounds where Xaa₁₄ is Leu, Ile, Val or pentylglycine, more preferably Leu or pentylglycine, and Xaa₂₅ is Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds will be less susceptible to oxidative degradation, both in vitro and in vivo, as well as during synthesis of the compound.

- Exendin agonist compounds also include those described in International Patent Application No. PCT/US98/24273, filed November 13, 1998, entitled, "Novel Exendin Agonist Compounds," which claims the benefit of United States Provisional Application No. 60/066,029, filed November 14, 1997, including compounds of the formula (III) [SEQ ID NO. 5]:

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀
Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁; wherein

- 5 Xaa₁ is His, Arg, Tyr, Ala, Norval, Val
or Norleu;
Xaa₂ is Ser, Gly, Ala or Thr;
Xaa₃ is Ala, Asp or Glu;
Xaa₄ is Ala, Norval, Val, Norleu or Gly;
10 Xaa₅ is Ala or Thr;
Xaa₆ is Phe, Tyr or naphthylalanine;
Xaa₇ is Thr or Ser;
Xaa₈ is Ala, Ser or Thr;
Xaa₉ is Ala, Norval, Val, Norleu, Asp or Glu;
15 Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;
Xaa₁₁ is Ala or Ser;
Xaa₁₂ is Ala or Lys;
Xaa₁₃ is Ala or Gln;
Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;
20 Xaa₁₅ is Ala or Glu;
Xaa₁₆ is Ala or Glu;
Xaa₁₇ is Ala or Glu;
Xaa₁₉ is Ala or Val;
Xaa₂₀ is Ala or Arg;
25 Xaa₂₁ is Ala or Leu;
Xaa₂₂ is Phe, Tyr or naphthylalanine;
Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine or
Met;
Xaa₂₄ is Ala, Glu or Asp;
30 Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;

Xaa₂₆ is Ala or Leu;

Xaa₂₇ is Ala or Lys;

Xaa₂₈ is Ala or Asn;

Z₁ is -OH,

5 -NH₂,

Gly-Z₂,

Gly Gly-Z₂,

Gly Gly Xaa₃₁-Z₂,

Gly Gly Xaa₃₁ Ser-Z₂,

10 Gly Gly Xaa₃₁ Ser Ser-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂,

15 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂ or

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈ Xaa₃₉-Z₂;

wherein

Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently

Pro, homoproline, 3Hyp, 4Hyp, thioproline,

20 N-alkylglycine, N-alkylpentylglycine or

N-alkylalanine; and

Z₂ is -OH or -NH₂;

provided that no more than three of Xaa₃, Xaa₄, Xaa₅,

Xaa₆, Xaa₈, Xaa₉, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆,

25 Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈

are Ala; and provided also that, if Xaa₁ is His, Arg or Tyr,

then at least one of Xaa₃, Xaa₄ and Xaa₅ is Ala.

Preparation of Compounds

The compounds that constitute active ingredients of the formulations and dosages of the present invention may be prepared using standard solid-phase peptide synthesis techniques and preferably an automated or semiautomated peptide synthesizer. The preparation of exendin-3 and exendin-4 is described in Examples 1 and 2 below. The preparation of additional exendin agonist peptide analogs is described in Examples 13-198 below.

Typically, using automated or semiautomated peptide synthesis techniques, an α -N-carbamoyl protected amino acid and an amino acid attached to the growing peptide chain on a resin are coupled at room temperature in an inert solvent such as dimethylformamide, N-methylpyrrolidinone or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in the presence of a base such as diisopropylethylamine. The α -N-carbamoyl protecting group is removed from the resulting peptide-resin using a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next desired N-protected amino acid to be added to the peptide chain. Suitable N-protecting groups are well known in the art, with t-butyloxycarbonyl (tBoc) and fluorenylmethoxycarbonyl (Fmoc) being preferred herein.

The solvents, amino acid derivatives and 4-methylbenzhydryl-amine resin used in the peptide synthesizer may be purchased from Applied Biosystems Inc. (Foster City, CA). The following side-chain protected amino acids may be purchased from Applied Biosystems, Inc.: Boc-Arg(Mts), Fmoc-Arg(Fmc), Boc-Thr(Bzl), Fmoc-Thr(t-Bu), Boc-Ser(Bzl), Fmoc-

Ser(t-Bu), Boc-Tyr(BrZ), Fmoc-Tyr(t-Bu), Boc-Lys(Cl-Z), Fmoc-Lys(Boc), Boc-Glu(Bzl), Fmoc-Glu(t-Bu), Fmoc-His(Trt), Fmoc-Asn(Trt), and Fmoc-Gln(Trt). Boc-His(BOM) may be purchased from Applied Biosystems, Inc. or Bachem Inc.

- 5 (Torrance, CA). Anisole, dimethylsulfide, phenol, ethanedithiol, and thioanisole may be obtained from Aldrich Chemical Company (Milwaukee, WI). Air Products and Chemicals (Allentown, PA) supplies HF. Ethyl ether, acetic acid, and methanol may be purchased from Fisher Scientific
10 (Pittsburgh, PA).

- Solid phase peptide synthesis may be carried out with an automatic peptide synthesizer (Model 430A, Applied Biosystems Inc., Foster City, CA) using the NMP/HOBt (Option 1) system and tBoc or Fmoc chemistry (see, Applied
15 Biosystems User's Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70, Applied Biosystems, Inc., Foster City, CA) with capping. Boc-peptide-resins may be cleaved with HF (-5 °C to 0°C, 1 hour). The peptide may be extracted from the resin with
20 alternating water and acetic acid, and the filtrates lyophilized. The Fmoc-peptide resins may be cleaved according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc., 1990, pp. 6-12). Peptides may also be assembled using an Advanced Chem Tech
25 Synthesizer (Model MPS 350, Louisville, Kentucky).

- Peptides may be purified by RP-HPLC (preparative and analytical) using a Waters Delta Prep 3000 system. A C4, C8 or C18 preparative column (10 µ, 2.2 x 25 cm; Vydac, Hesperia, CA) may be used to isolate peptides, and purity
30 may be determined using a C4, C8 or C18 analytical column (5

μ , 0.46 x 25 cm; Vydac). Solvents (A=0.1% TFA/water and B=0.1% TFA/CH₃CN) may be delivered to the analytical column at a flow rate of 1.0 ml/min and to the preparative column at 15 ml/min. Amino acid analyses may be performed on the

5 Waters Pico Tag system and processed using the Maxima program. Peptides may be hydrolyzed by vapor-phase acid hydrolysis (115°C, 20-24 h). Hydrolysates may be derivatized and analyzed by standard methods (Cohen, et al., The Pico Tag Method: A Manual of Advanced Techniques for Amino Acid

10 Analysis, pp. 11-52, Millipore Corporation, Milford, MA (1989)). Fast atom bombardment analysis may be carried out by M-Scan, Incorporated (West Chester, PA). Mass calibration may be performed using cesium iodide or cesium iodide/glycerol. Plasma desorption ionization analysis

15 using time of flight detection may be carried out on an Applied Biosystems Bio-Ion 20 mass spectrometer. Electrospray mass spectroscopy may be carried and on a VG-Trio machine.

Peptide active ingredient compounds useful in the

20 formulations and dosages of the invention may also be prepared using recombinant DNA techniques, using methods now known in the art. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor (1989).

25 Utility

The formulations and dosages described herein are useful in view of their pharmacological properties. In particular, the formulations and dosages of the invention are effective as exendins and exendin agonists, and possess

30 activity as agents to lower blood glucose, and to regulate

gastric motility and to slow gastric emptying, as evidenced by the ability to reduce post-prandial glucose levels in mammals.

5 Formulation and Administration

Exendin and exendin agonist formulations and dosages of the invention are useful in view of their exendin-like effects, and may conveniently be provided in the form of formulations suitable for parenteral (including intravenous, 10 intramuscular and subcutaneous) administration. Also described herein are formulations and dosages useful in alternative delivery routes, including oral, nasal, buccal, sublingual and pulmonary.

Compounds useful in the invention can be provided as 15 parenteral compositions for injection or infusion. Generally, they can, for example, be suspended in an inert oil, suitably a vegetable oil such as sesame, peanut, olive oil, or other acceptable carrier. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic 20 buffer solution at a pH of about 3.0 to about 7.0, more specifically from about 4.0 to 6.0, and preferably from about 4.0 to about 5.0. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain 25 pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents. Useful buffers include for example, sodium acetate/acetic acid buffers. The desired isotonicity may be accomplished using sodium chloride or other 30 pharmaceutically acceptable agents such as dextrose, boric

acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

5 The exendin and exendin agonist compounds can also be formulated as pharmaceutically acceptable salts (e.g., acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts
10 can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical
15 transmucosal administration and increasing the solubility to facilitate the administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride,
20 phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids
25 such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethane sulfonic acid, benzene sulfonic acid, *p*-toluenesulfonic acid, cyclohexyl sulfamic acid, and quinic acid. Such salts
30 may be prepared by, for example, reacting the free acid or base forms of the product with one or more equivalents of

the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a
5 suitable ion exchange resin.

Generally, carriers or excipients can also be used to facilitate administration of the dosages of the present invention. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as
10 lactose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

If desired, solutions of the above dosage compositions may be thickened with a thickening agent such as
15 methylcellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for example, acacia powder, a non-ionic surfactant (such as a Tween), or an ionic surfactant (such
20 as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

In general, formulations and dosage compositions of the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected
25 components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control
30 tonicity.

Other pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises, e.g., Remington's Pharmaceutical Sciences by E.W. Martin.

- See also Wang, Y.J. and Hanson, M.A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988).

For use by the physician, the compounds will be provided in dosage unit form containing an amount of an
10 exendin agonist, with or without another therapeutic agent, for example, a glucose-lowering agent, a gastric emptying modulating agent, a lipid lowering agent, or a food intake inhibitor agent. Therapeutically effective amounts of an exendin agonist for use in the control of blood glucose or
15 in the control of gastric emptying and in conditions in which gastric emptying is beneficially slowed or regulated are those that decrease post-prandial blood glucose levels, preferably to no more than about 8 or 9 mM or such that blood glucose levels are reduced as desired. In diabetic or
20 glucose intolerant individuals, plasma glucose levels are higher than in normal individuals. In such individuals, beneficial reduction or "smoothing" of post-prandial blood glucose levels may be obtained. As will be recognized by those in the field, an effective amount of therapeutic agent
25 will vary with many factors including the patient's physical condition, the blood sugar level or level of inhibition of gastric emptying to be obtained, or the desired level of food intake reduction, and other factors.

Such pharmaceutical compositions are useful in causing
30 increased insulin sensitivity in a subject and may be used

as well in disorders, such as diabetes, where sensitivity to insulin is beneficially increased.

A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or other form of delivery.

The effective daily doses of the compounds are described. The exact dose to be administered may be determined by the attending clinician and may be further dependent upon the efficacy of the particular exendin or exendin agonist compound used, as well as upon the age, weight and condition of the individual. The optimal mode of administration of compounds of the present application to a patient depend on factors known in the art such as the particular disease or disorder, the desired effect, and the type of patient. While the compounds will typically be used to treat human patients, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and poultry, and sports animals and pets such as horses, dogs and cats.

The invention includes formulations of exendins and exendin agonists that comprise an exendin or exendin agonist mixed together with a buffer (preferably an acetate buffer), an iso-osmolality modifier (preferably mannitol), and optionally containing a preservative (preferably m-cresol), said formulation having a pH of between about 3.0 and about 7.0 (preferably between about 4.0 and about 5.0).

The formulation which best supports a parenteral liquid dosage form is one in which the active ingredient(s) is stable with adequate buffering capacity to maintain the pH of the solution over the intended shelf life of the product.

- 5 The dosage form should be either an isotonic and/or an iso-osmolar solution to either facilitate stability of the active ingredient or lessen the pain on injection or both. Devices that deliver very small injection volumes, however, may not require that the formulation be either isotonic
10 and/or iso-osmolar. If the dosage form is packaged as a unit-dose, then a preservative may be included but is not required. If, however, the dosage form is packaged in a multi-use container, then a preservative is necessary.

- These dosage forms include approximately 0.005 to about
15 0.4%, more specifically from about 0.005 to about 0.02%, or from about 0.005 to about 0.05% (w/v), respectively of the active ingredient in an aqueous system along with approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate or similar buffer either alone or in
20 combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0, as well as either approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol iso-osmolality modifier (preferably
25 mannitol) or up to about 0.9% saline or a combination of both leading to an isotonic or an iso-osmolar solution in an aqueous continuous phase. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl ethyl, propyl
30 and butyl parabens and phenol is also present if the

formulation is packaged in a multi-use container. A sufficient amount of water for injection is added to obtain the desired concentration of solution. Sodium chloride, as well as other excipients, may also be present, if desired.

- 5 Such excipients, however, must maintain the overall stability of the active ingredient.

Polyhydric alcohols and carbohydrates share the same feature in their backbones, i.e., -CHOH-CHOH-. The polyhydric alcohols include such compounds as sorbitol, mannitol, glycerol, and polyethylene glycols (PEGs). These compounds are straight-chain molecules. The carbohydrates, such as mannose, ribose, trehalose, maltose, glycerol, inositol, glucose and lactose, on the other hand, are cyclic molecules that may contain a keto or aldehyde group. These two classes of compounds will also be effective in stabilizing protein against denaturation caused by elevated temperature and by freeze-thaw or freeze-drying processes. Suitable carbohydrates include galactose, arabinose, lactose or any other carbohydrate which does not have an adverse affect on a diabetic patient, i.e., the carbohydrate is not metabolized to form large concentrations of glucose in the blood. Such carbohydrates are well known in the art as suitable for diabetics.

Preferably, the peptides of the present invention are admixed with a polyhydric alcohol such as sorbitol, mannitol, inositol, glycerol, xylitol, and polypropylene/ethylene glycol copolymer, as well as various polyethylene glycols (PEG) of molecular weight 200, 400, 1450, 3350, 4000, 6000, and 8000). Mannitol is the preferred polyhydric alcohol.

The liquid formulation of the invention should be substantially isotonic and/or iso-osmolar. An isotonic solution may be defined as a solution that has a concentration of electrolytes, or a combination of

5 electrolytes and non-electrolytes that will exert equivalent osmotic pressure as that into which it is being introduced, here for example in the case of parenteral injection of the formulation, a mammalian tissue. Similarly, an iso-osmolar solution may be defined as a solution that has a

10 concentration of non-electrolytes that will exert equivalent osmotic pressure as that into which it is being introduced. As used herein, "substantially isotonic" means within $\pm 20\%$ of isotonicity, preferably within $\pm 10\%$. As used herein, "substantially iso-osmolar" means within $\pm 20\%$ of iso-

15 osmolality, preferably within $\pm 10\%$. The formulated product for injection is included within a container, typically, for example, a vial, cartridge, prefilled syringe or disposable pen.

The formulation which best support a unit-dose

20 parenteral lyophilized dosage form is one in which the active ingredient is reasonably stable, with or without adequate buffering capacity to maintain the pH of the solution over the intended shelf life of the reconstituted product. The dosage form should contain a bulking agent to

25 facilitate cake formation. The bulking agent may also act as a tonicifer and/or iso-osmolality modifier upon reconstitution to either facilitate stability of the active ingredient and/or lessen the pain on injection. As noted above, devices that deliver very small injection volumes may

30 not require the formulation to be isotonic and/or iso-

osmolar. A surfactant may also benefit the properties of the cake and/or facilitate reconstitution.

These dosage forms include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or 5 0.005 to 0.05% (w/v) of the active ingredient. It may not be necessary to include a buffer in the formulation and/or to reconstitute the lyophile with a buffer if the intention is to consume the contents of the container within the stability period established for the reconstituted active 10 ingredient. If a buffer is used, it may be included in the lyophile or in the reconstitution solvent. Therefore, the formulation and/or the reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer 15 either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol iso- 20 osmolality modifier (as described above) or up to 0.9% saline or a combination of both leading to a isotonic or iso-osmolar solution in the reconstituted aqueous phase. A surfactant, preferably about 0.1 to about 1.0% (w/v) of polysorbate 80 or other non-ionic detergent, may be 25 included. As noted above, sodium chloride, as well as other excipients, may also be present in the lyophilized unit-dosage formulation, if desired. Such excipients, however, must maintain the overall stability of the active ingredient. The formulation will be lyophilized within the

validation parameters identified to maintain stability of the active ingredient.

The liquid formulation of the invention before lyophilization should be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation of isotonic and/or iso-osmolar solutions after reconstitution. The formulation should be used within the period established by shelf-life studies on both the lyophilized form and following reconstitution. The lyophilized product is included within a container, typically, for example, a vial. If other containers are used such as a cartridge, pre-filled syringe, or disposable pen, the reconstitution solvent may also be included.

As with the parenteral liquid and lyophilized unit-dosage formulations described above, the formulation which best supports a multi-dose parenteral lyophilized dosage form is one in which the active ingredient is reasonably stable with adequate buffering capacity to maintain the pH of the solution over the intended "in-use" shelf-life of the product. The dosage form should contain a bulking agent to facilitate cake formation. The bulking agent may also act as a tonicifer and/or iso-osmolality modifier upon reconstitution to either facilitate stability of the active ingredient or lessen the pain on injection or both. Again, devices that deliver very small injection volumes may not require the formulation to be either isotonic and/or iso-osmolar. A preservative is, however, necessary to facilitate multiple use by the patient.

These dosage forms include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or

from about 0.005 to 0.05% (w/v), respectively of the active ingredient. It may not be necessary to include a buffer in the formulation and/or to reconstitute the lyophile with a buffer if the intention is to consume the contents of the container within the stability period established for the reconstituted active ingredient. If a buffer is used, it may be included in the lyophile or in the reconstitution solvent. Therefore, the formulation and/or the reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either approximately 1.0 to 10% (w/v) of a carbohydrate or a polyhydric alcohol iso-osmolality modifier (preferably mannitol) or up to 0.9% saline, or a combination of both, leading to an isotonic or iso-osmolar solution in the reconstituted aqueous phase. A surfactant, preferably about 0.1 to about 1.0% (w/v) of polysorbate 80 or other non-ionic detergent, may be included. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol (preferably m-cresol) is also present if the formulation is packaged in a multi-use container. Sodium chloride, as well as other excipients, may also be present, if desired. Again, however, such excipients must maintain the overall stability of the active ingredient. The formulation should be lyophilized within the validation

parameters identified to maintain stability of the active ingredient. The liquid formulation of the invention should be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation of isotonic and/or iso-osmolar solutions after reconstitution. The formulation should be used within the period established by shelf-life studies on both the lyophilized form and following reconstitution. The lyophilized product is included within a container, typically, for example, a vial. If other containers are used such as a cartridge, pre-filled syringe or disposable pen, the reconstitution solvent may also be included.

The formulations that best support oral, nasal, pulmonary and/or intra-tracheal dosage forms may be either preserved or unpreserved liquid formulations and/or dry powder or, for oral administration, solid formulations. The preserved or unpreserved liquid formulations will be essentially identical to the formulations described above under preserved or unpreserved liquid parenteral formulations. The pH of the solution should be about 3.0 to 7.0, with a pH greater than or equal to about 5.0 being most preferred to reduce the potential for bronchoconstriction. The dry powder formulations may contain a bulking agent and/or salts to facilitate particle size formation and appropriate particle size distribution. A surfactant and/or salts may also benefit the properties of the particle morphology and/or facilitate tissue uptake of the active ingredient.

These dry powder dosage forms can range from 1% to 100% (w/w), respectively of the active ingredient. It may not be

necessary to include a bulking agent and/or salts to facilitate particle size formation and/or distribution. The bulking agent and/or salts may consist of either approximately 0 to 99% (w/w) of a carbohydrate or polyhydric alcohol or approximately 0 to 99% salt or a combination of both leading to the preferred particle size and distribution. A surfactant, preferably about 0.1 to about 1.0% (w/w) of polysorbate 80 or other non-ionic detergent, may be included. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, must maintain the overall stability of the active ingredient and facilitate the proper level of hydration.

The formulations that best support nasal and/or intra-tracheal dosage forms may be either preserved or unpreserved liquid dosage formulations described previously.

Dissolvable gels and/or patches may be used to facilitate buccal delivery. The gels may be prepared from various types of starch and/or cellulose derivatives.

Sublingual delivery may be best supported by liquid formulations similar to those described above as parenteral liquid or parenteral lyophilized formulations after reconstitution except without the need for the dosage form to be an isotonic and/or iso-osmolar solution. Solid dosage forms may be similar to oral solid dosage forms except that they must be readily dissolvable under the tongue.

Oral delivery may be best supported by a liquid (gel cap) formulation that is similar to the parenteral liquid formulation except that the solution may be more concentrated and may contain additional additives to

facilitate uptake of the active ingredient by the small intestine. Solid dosage forms will contain inert ingredients along with the active ingredient to facilitate tablet formation. These ingredients may include polyhedral alcohols (such as mannitol), carbohydrates, or types of starch, cellulose derivatives, and/or other inert, physiologically compatible materials. The tablet may be enterically coated to minimize digestion in the stomach and thereby facilitate dissolution and uptake further along the alimentary canal.

The invention also includes preferred dosages for exendins and exendin agonists when given by injection, and when given by other routes. Thus, formulations for exendin and exendin agonists having comparable potency are prepared for the administration by injection and include from about 0.1 to about 0.5 μg per kilogram, given one to three times per day. Typically, for the patient with diabetes who weighs in the range from about 70 kilograms (average for the type 1 diabetic) to about 90 kilograms (average for the type 2 diabetic), for example, this will result in the total administration of about 10 to about 120 μg per day in single or divided doses. If administered in divided doses, the doses are preferably administered two or three times per day, and more preferably, two times per day.

In a preferred injection procedure, the exendin or exendin agonist is administered parenterally, more preferably by injection, for example, by peripheral injection. Preferably, about 1 μg -30 μg to about 1 mg of the exendin or exendin agonist is administered per day. More preferably, about 1-30 μg to about 500 μg , or about 1-30 μg to about 50

μg of the exendin or exendin agonist is administered per day. Most preferably, depending upon the weight of the subject and the potency of the compound administered, about 3 μg to about 50 μg of the exendin or exendin agonist is administered per day. Preferred doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.005 $\mu\text{g}/\text{kg}$ per dose to about 0.2 $\mu\text{g}/\text{kg}$ per dose. More preferably, doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.02 $\mu\text{g}/\text{kg}$ per dose to about 0.1 $\mu\text{g}/\text{kg}$ per dose. Most preferably, doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.05 $\mu\text{g}/\text{kg}$ per dose to about 0.1 $\mu\text{g}/\text{kg}$ per dose. These doses are administered from 1 to 4 times per day, preferably from 1 to 2 times per day. Doses of exendins or exendin agonists will normally be lower if given by continuous infusion. Doses of exendins or exendin agonists will normally be higher if given by non-injection methods, such as oral, buccal, sublingual, nasal, pulmonary or skin patch delivery.

Oral dosages according to the present invention will include from about 50 to about 100 times the active ingredient, i.e., from about 500 to about 12,000 μg per day in single or divided doses, preferably from about 500 to about 5,000 μg per day. Pulmonary dosages according to the present invention will include from about 10 to about 100 times the active ingredient, i.e., from about 100 to about 12,000 μg per day in single or divided doses, preferably about 500 to 1000 μg per day. Nasal, buccal and sublingual

dosages according to the present invention will also include from about 10 to about 100 times the active ingredient, i.e., from about 100 to about 12,000 μg per day in single or divided doses.

- 5 Preferred dosages for nasal administration are from about 10-1000 to about 1200-12,000 μg per day, for buccal administration from about 10-1000 to about 1200-12,000 μg per day, and for sublingual administration from about 10-1000 to about 1200-8,000 μg per day. Sublingual dosages are preferably smaller than buccal dosages. Administration dosages for exendin agonists having less than or greater than the potency of exendin-4 are increased or decreased as appropriate from those described above and elsewhere herein.

15 Clinical Studies

- As described in Example 10 below, a double blind, placebo-controlled single ascending dose study examining the safety, tolerability, and pharmacokinetics of subcutaneous exendin-4 in healthy volunteers has been completed. Five single subcutaneous doses of exendin-4 (0.01, 0.05, 0.1, 0.2 or 0.3 $\mu\text{g}/\text{kg}$) were studied in 40 healthy male volunteers in the fasting state. Maximum plasma exendin-4 concentrations were achieved between one and two hours post-dose with little difference among the doses examined. Examination of the data indicated a dose dependent increase for C_{max} . There were no serious adverse events reported in this study.

In the healthy male volunteers that participated in this study, exendin-4 was well tolerated at subcutaneous doses up to and including 0.1 $\mu\text{g}/\text{kg}$. A decrease in plasma

glucose concentration was also observed at this dose. At doses of 0.2 $\mu\text{g/kg}$ and higher, the most commonly observed adverse events were headache, nausea, vomiting, dizziness, and postural hypotension. There was a transient fall in plasma glucose concentration following administration of doses of 0.05 $\mu\text{g/kg}$ and above.

Example 12 below describes a further study of the dose-response relationship for the glucose-lowering effect of exendin-4 at doses less than 0.1 $\mu\text{g/kg}$. Fourteen subjects [mean ($\pm\text{SE}$) age 55 ± 2 ; mean BMI ($30.2 \pm 1.6 \text{ kg/m}^2$)] with type 2 diabetes treated with diet \pm oral hypoglycemic agents were studied following withdrawal of oral agents for 10-14 days. Assessments were made following randomized, subcutaneous injection of placebo, 0.01, 0.02, 0.05 and 0.1 $\mu\text{g/kg}$ exendin-4 on separate days following an overnight fast. Injections were given immediately before ingestion of a standardized Sustacal® meal (7kcal/kg) followed by collection of plasma glucose samples at frequent intervals during the subsequent 300 minutes.

The glycemic response was quantified as the time-weighted mean ($\pm\text{SE}$) change in plasma glucose concentration during the 5-hr period. The response ranged from a $+42.0 \pm 7.9 \text{ mg/dL}$ increment above the fasting glucose concentration for placebo compared to a $30.5 \pm 8.6 \text{ mg/dL}$ decrement below the fasting glucose concentration with 0.1 $\mu\text{g/kg}$ exendin-4.

The ED_{50} for this glucose lowering effect was 0.038 $\mu\text{g/kg}$. Exendin-4 doses less than 0.1 $\mu\text{g/kg}$ appeared to disassociate the glucose lowering effects from the gastrointestinal side effects. Example 12 shows that

exendin-4 was not only well tolerated at doses less than 0.1 $\mu\text{g/kg}$, but that these doses substantially lowered postprandial plasma glucose concentrations (ED_{50} of 0.038 $\mu\text{g/kg}$) in people with type 2 diabetes.

5

Alternate Routes of Delivery

The feasibility of alternate routes of delivery for exendin-4 has been explored by measuring exendin-4 in the circulation in conjunction with observation of a biologic response, such as plasma glucose lowering in diabetic animals, after administration. Passage of exendin-4 has been investigated across several surfaces, the respiratory tract (nasal, tracheal, and pulmonary routes) and the gut (sublingual, gavage and intraduodenal routes). Biologic effect and appearance of exendin-4 in blood have been observed with each route of administration via the respiratory tract, and with sublingual and gavaged peptide via the gastrointestinal tract.

Intra-tracheal Administration - As described herein, intra-tracheal administration of exendin-4 into fasted rats (20 $\mu\text{g}/50\mu\text{L}/\text{animal}$) produced a rise in the mean plasma exendin-4 concentration to 2060 ± 960 pg/mL within 5-10 minutes after administration. Elevated plasma exendin-4 concentrations were maintained for at least 1 hour after instillation (see Figure 4). In diabetic *db/db* mice, intra-tracheal instillation of exendin-4 (1 $\mu\text{g}/\text{animal}$) lowered plasma glucose concentration by 30% while that in the vehicle control group increased by 41% 1.5 hours after treatment. In these animals the mean plasma concentration

of exendin-4 was 777 ± 365 pg/ml at 4.5 hours after treatment (see Figures 5a and 5b).

In diabetic *ob/ob* mice, intra-tracheal instillation of exendin-4 (1 μ g/animal) decreased plasma glucose concentration to 43% of the pre-treatment level after 4 hours while that in the vehicle control group was not changed (see Figures 6a and 6b).

Nine overnight-fasted male Sprague Dawley rats (age 96-115 days, weight 365-395, mean 385g) were anesthetized with halothane, tracheotomized, and catheterized via the femoral artery. At $t=0$ min, 30 μ L of saline in which was dissolved 2.1 μ g ($n=3$), 21 μ g ($n=3$) or 210 μ g of exendin-4 was instilled into the trachea beyond the level of intubation. Blood samples were taken after 5, 10, 20, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min, centrifuged and plasma stored at -20°C for subsequent immunoradiometric (IRMA) assay directed to N-terminal and C-terminal epitopes of the intact exendin-4 molecule. Following intra-tracheal administration, 61-74% of peak plasma concentration was observed within 5 min. T_{max} occurred between 20 and 30 min after administration. AUC and C_{max} were proportional to dose. At a dose of 2.1 μ g (1.5 nmol/kg), resulting in plasma concentrations of ~ 50 pM (where glucose-lowering effects in man are observed), bioavailability was 7.3%. The coefficient of variation was 44%. At higher doses, bioavailability was slightly lower, and the CV was higher (see Figures 7a and 7b). Via the tracheal route of administration, the $t_{1/2}$ (defined pragmatically as time for plasma to fall below 50% of C_{max}) was 30-60 min for the lowest dose and 60-90 min for the 2 higher doses. In sum, biologically effective quantities of

exendin-4 are rapidly absorbed via the trachea without evoking apparent respiratory distress. The respiratory tract is a viable route of administration of exendin-4.

Pulmonary Administration - Increased plasma

- 5 concentrations of exendin-4 were detected in rats exposed to aerosolized exendin-4. Exposure of rats to approximately 8 ng of aerosolized exendin-4 per mL of atmosphere for 10 minutes resulted in peak plasma exendin-4 concentrations of 300-1900 pg/mL 5 minutes following treatment (see Figure 8).
- 10 Similar exposure of diabetic *db/db* mice to aerosolized exendin-4 lead to a 33 % decrease in plasma glucose concentration after 1 hour, when a mean plasma exendin-4 concentration of 170 ± 67 pg/mL was detected. Diabetic *db/db* mice in the control group exposed to aerosolized
- 15 saline recorded no change in plasma glucose (see Figures 9a and 9b).

- Nasal administration - Application of exendin-4 into the nasal cavity of rats led to a rise in plasma concentrations. Peak values of 300 pg/mL and 6757 pg/mL*
- 20 *were detected 10 minutes after administration of 1 μ g and 100 μ g exendin-4 (dissolved in 2 μ L saline), respectively (see Figure 10).*

- Administration via the Gut- Male db/db mice (approximately 50g body wt.) were fasted for 2h and before*
- 25 *and after an intra-gastric administration of saline or exendin-4 (exendin-4). A 9% decrease in plasma glucose concentration was observed with 1mg/200 μ l/animal and a 15% decrease was observed with 3 mg/200 μ l/animal, compared with a 10% increase plasma glucose in the controls one hour after*
- 30 *treatment (see Figure 11).*

Sublingual Administration - Sublingual application of exendin-4 (100 $\mu\text{g}/5 \mu\text{L}/\text{animal}$) to diabetic *db/db* mice led to a 15% decrease in plasma glucose concentration one hour after treatment. A 30% increase was observed for the control group receiving saline. The mean exendin-4 plasma level at 60 minutes was $4520 \pm 1846 \text{ pg/mL}$ (see Figures 12a, 12b, and 12c).

Eight Sprague Dawley rats (~300g) were briefly anesthetized with metophane while a solution containing 10 $\mu\text{g}/3 \mu\text{L}$ (n=4) or 100 $\mu\text{g}/3 \mu\text{L}$ (n=4) was pipetted under the tongue. Blood samples were subsequently collected from the topically anesthetized tail and assayed for exendin-4 by IRMA. Plasma concentrations had begun to rise by 3 min after administration and were maximal 10 min and 30 min after administration (10 μg and 100 μg doses, respectively). Plasma exendin-4 concentration subsequently remained above the lower limit of quantitation (LLOQ) beyond 5 hours. Area-under-the-curve to the end of each experiment was calculated by the trapezoidal method. Two numbers were derived, one derived from total immunoreactivity, the other derived from the increment above the non-zero value present at t=0. These values were compared to historical intravenous bolus data in the same animal model to obtain, respectively, high and low estimates of bioavailability. For the 10 μg dose, sublingual bioavailability was 3.1-9.6%, and for a 100 μg dose, bioavailability was lower at 1.3-1.5%. Variability of AUC was greatest in the first hour after administration (CV 74% and 128% for 10 and 100 μg doses). For the 5-hour integral, coefficient of variation of the AUC was 20% and 64%, respectively. Peak plasma concentration

(C_{max}) occurred as rapidly after sublingual administration as after subcutaneous administration (T_{max} ~30 min). C_{max} after sublingual administration of 10 µg exendin-4 was 1.5% that after an intravenous bolus, but 14.5% of that obtained after a subcutaneous bolus. C_{max} after sublingual administration of 100 µg exendin-4 was only 0.29% of that observed after an intravenous bolus, and 6.1% of that obtained after a subcutaneous bolus (see Figures 12d and 12e). Thus, exendin-4 can be delivered at bioeffective doses via the sublingual route. Bioavailability and C_{max} were greatest, T_{max} was shortest, and variability of availability was least with the lowest sublingual dose. The lowest sublingual dose resulted in plasma concentrations similar to those that are predicted to be effective in lowering glucose in humans (~50-100 pM).

To assist in understanding the present invention the following Examples are included which describe the results of a series of experiments. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

EXAMPLE 1 - PREPARATION OF EXENDIN-3

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 1]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. Deprotection (Fmoc group removal) of the growing peptide chain was achieved using piperidine. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 75%.

Used in purification steps and analysis of Examples 1 and 2 were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.2 minutes.

EXAMPLE 2 - PREPARATION OF EXENDIN-4

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 2]

- 5 The above amidated peptide was assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
10 similar way to Exendin-3 as describe in Example 1. Used in
analysis were Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide gave product peptide having an observed retention
15 time of 14.9 minutes. Electrospray Mass Spectrometry (M):
calculated 4186.6; found 8186.0 to 4186.8 (four lots).

EXAMPLE 3 - Exendin-4 IS A CIRCULATING,
MEAL-RELATED PEPTIDE IN THE GILA MONSTER

- 20 This experiment investigated whether exendin-4 has a
metabolic role in the Gila monster lizard itself. To
investigate whether exendin-4 appeared in the blood of the
Gila monster in response to feeding, blood was sampled from
one animal fasted for 7 weeks, before and 30 min after
25 ingestion of a small rat. Plasma was assayed for full-
length exendin-4 using an immunoradiometric assay with
monoclonal antibody pairs directed to epitopes at N- and
C-termini of exendin-4. Fasting plasma exendin-4
concentration was 76 pg/mL, near the lower limit of

quantitation. After eating, this value rose 300-fold to 23,120 pg/mL.

In a second experiment, serial samples were taken from two animals fasted five weeks before and after ingestion of one or two small rats (47-49 g). Plasma exendin-4 concentration rose 23- to 36-fold (to 4860, 8340 pg/mL) immediately after eating, consistent with a direct passage of exendin-4 from the salivary gland to blood. After eating a second rat ($t=30$ min), plasma exendin-4 concentration in one Gila rose further to 27,209 pg/mL. Plasma exendin-4 concentration decayed with a $t_{1/2}$ of 5.00 and 5.33 hours, respectively. In conclusion, exendin-4, known to originate from the salivary gland of the Gila monster, appears in high concentration in the blood immediately after eating. This may represent a meal-related signal to inhibit further eating and promote nutrient storage.

EXAMPLE 4 - EXENDIN-4 DECREASES GLUCAGON SECRETION DURING HYPERGLYCEMIC CLAMPS IN DIABETIC FATTY ZUCKER RATS

Absolute or relative hyperglucagonemia is often a feature of type 1 and type 2 diabetes mellitus, and the suppression of excessive glucagon secretion is a potential benefit of therapy using glucagonostatic agents. In this Example, the effect of exendin-4 on glucagon secretion in male anaesthetized Diabetic Fatty Zucker (ZDF) rats was examined. Using an hyperinsulinemic hyperglycemic clamp protocol, factors tending to influence glucagon secretion were held constant. Plasma glucose was clamped at ~ 34 mM 60 min before beginning intravenous infusions of saline ($n=7$) or exendin-4 ($0.21 \mu\text{g} + 2.1 \mu\text{g/mL/h}$; $n=7$). Plasma glucagon

concentration measured before these infusions were similar in both groups ($306 \pm 30\text{pM}$ versus $252 \pm 32\text{pM}$, respectively; n.s.).

Mean plasma glucagon concentration in exendin-4 infused rats was nearly half of that in saline-infused rats in the final 60 minutes of the clamp ($165 \pm 18\text{pM}$ versus $298 \pm 26\text{pM}$, respectively; $P < 0.002$). The hyperglycemic clamp protocol also enabled measurement of insulin sensitivity. Glucose infusion rate during the clamp was increased by $111 \pm 7\%$ in exendin-4-treated versus control rats ($P < 0.001$). In other words, exendin-4 exhibited a glucagonostatic effect in ZDF rats during hyperglycemic clamp studies, an effect that will be of therapeutic benefit in diabetic humans.

EXAMPLE 5 - PHARMACOKINETICS OF EXENDIN-4

IN THE RAT FOLLOWING INTRAVENOUS,

SUBCUTANEOUS AND INTRAPERITONEAL ADMINISTRATION

This Example describes work to define the plasma pharmacokinetics of exendin-4 in rats (~350g body weight each) following 2.1, 21, 210 $\mu\text{g}/\text{rat}$ i.v. bolus, s.c. and i.p. administration and 2.1, 21, 210 $\mu\text{g}/\text{hr}/\text{rat}$ i.v. infusion (3 hr). Serial samples of plasma (~120 μL) were assayed using a validated immunoradiometric assay (IRMA). This sandwich-type assay uses mouse-based monoclonal antibodies that react with exendin-4 but do not react with GLP-1 or tested metabolites of exendin-4 or GLP-1. The lower limit of quantitation was 15pM (63pg/mL). The estimated $t_{1/2}$ for exendin-4 was 18-41 min for i.v. bolus, 28-49 for i.v. continuous, 90-216 min for s.c. and 125-174 min for i.p. injection. Bioavailability was 65-76% for s.c. and i.p.

- injection. Clearance determined from the i.v. infusion was 4-8 mL/min. Both C_{\max} and AUC values within each route of administration were proportional to dose. Volume of distribution was 457-867 mL. Clearance and bioavailability were not dose dependent. C_{\max} (or steady-state plasma concentration; C_{ss}) is shown in the table below

Cmax or C _{ss} (nM)				
Route	Intravenous bolus	Intravenous infusion	Subcutaneous	Intraperitoneal
Dose				
2.1 μg	2.9 \pm 0.4	1.1 \pm 0.1	0.56 \pm 0.12	0.26 \pm 0.04
21 μg	70 \pm 3	19 \pm 1.9	4.1 \pm 1.5	3.9 \pm 1
210 μg	645 \pm 12	262 \pm 60	28 \pm 4	35 \pm 6

10 EXAMPLE 6 - COMPARISON OF THE INSULINOTROPIC ACTIONS
OF EXENDIN-4 AND GLUCAGON-LIKE PEPTIDE-1 (GLP-1)
DURING AN INTRAVENOUS GLUCOSE CHALLENGE IN RATS

- This experiment compares the insulinotropic actions of synthetic exendin-4 and GLP-1 in vivo following an intravenous (i.v.) glucose challenge in rats. Sprague-Dawley rats (~400g) were anesthetized with halothane and cannulated via the femoral artery and saphenous vein. Following a 90-min recovery period, saline or peptide (30 pmol/kg/min each) was administered i.v. (1ml/h for 2 hours; n=4-5 for each group). Thirty min after infusion commenced, D-glucose (5.7mmol/kg, 0.8ml) was injected i.v. In saline-treated, exendin-4-treated and GLP-1-treated rats, plasma glucose concentrations were similar before injection (9.3 \pm 0.3, 9.7 \pm 0.3, 10.3 \pm 0.4mM), increased by similar amounts

after glucose injection (21.7, 21.3, 23.7mM), and resulted in a similar 60-min glucose AUC (987 ± 39 , 907 ± 30 , $1096 \pm 68 \text{ mM} \cdot \text{min}$, respectively). That is, the glycemic stimulus was similar in each treatment group. Plasma insulin concentration in saline-treated rats increased 3.3-fold with the glucose challenge (230 ± 53 to a peak of $765 \pm 188 \text{ pM}$). With exendin-4 infusion, the increase in plasma insulin concentration was 6.8-fold (363 ± 60 to $2486 \pm 365 \text{ pM}$). With GLP-1 the increase in plasma insulin concentration was 2.9-fold (391 ± 27 to $1145 \pm 169 \text{ pM}$), which was similar to that obtained in saline-treated rats. The 60-min insulin AUC in saline-treated rats was $24 \pm 6 \text{ nM} \cdot \text{min}$, was increased 2.8-fold in exendin-treated rats ($67 \pm 8 \text{ nM} \cdot \text{min}$; $P < 0.003$ versus saline; $P < 0.02$ versus GLP-1) and by 20% in GLP-1-treated rats (n.s. versus saline). Amplification of glucose-stimulated insulin release by exendin-4 was also tested at infusion rates of 3 and 300 pmol/kg/min and shown to be dose-dependent. Thus, exendin-4 is more potent and/or effective than GLP-1 in amplifying glucose-stimulated insulin release in intact rats.

EXAMPLE 7 - DEVELOPMENT AND VALIDATION OF AN IMMUNORADIOMETRIC ASSAY (IRMA) FOR THE QUANTITATION OF EXENDIN-4 IN PLASMA AND ITS APPLICATION TO PRECLINICAL TOXICITY AND PHASE I CLINICAL EVALUATIONS

A sensitive and specific sandwich-type immunoradiometric (IRMA) assay was developed for quantitation of plasma exendin-4 concentration using synthetic exendin-4 as the immunogen. One mouse-derived monoclonal antibody recognizes

a C-terminal epitope on exendin-4 (capture antibody) but does not cross-react with GLP-1. The second antibody (detector antibody labeled with ^{125}I) recognizes an N-terminal epitope on exendin-4 and GLP-1, and requires a terminal histidine for binding. Thus, the assay as a whole does not detect GLP-1(7-36)NH₂, GLP-1(7-36)COOH or exendin(3-39). Assay validation in rat, monkey, dog, rabbit and human plasmas showed inter- and intra-assay coefficients of variation <20% and <10%, respectively, accuracy of $\pm 15\%$ with target low, mid and high controls, and lower and upper limits of quantitation of 62.8 and 2512 pg/mL, respectively. Plasma samples from 28-day subcutaneous toxicity evaluations of exendin-4 in rats and monkeys and a Phase I clinical study in normal subjects were evaluated using the IRMA. The C_{max} values in the animals studies are shown in the table below. Human samples from subcutaneous administration of 0.05, 0.1, 0.2 and 0.3 $\mu\text{g/kg}$ yielded C_{max} values of 90, 224, 370 and 587 pg/mL.

C_{max} (pg/mL)			
Dose ($\mu\text{g/kg}$)	10	100	1000
Rat	7,000	127,000	1,180,000
Monkey	20,000	170,000	1,890,000

EXAMPLE 8 - COMPARISON OF GLP-1 RECEPTOR BINDING/ACTIVATING AND GLUCOSE-LOWERING EFFECTS OF GLP-1 AND EXENDIN-4

Exendin-4 was synthesized by solid phase peptide synthesis techniques and compared to synthetic GLP-1 in terms of *in vitro* binding to, and activation of, GLP-1

receptors, and *in vivo* in terms of lowering plasma glucose in diabetic *db/db* mice. In a plasma membrane preparation of a rat insulinoma cell line (RINm5f) that expresses the GLP-1 receptor, the peptides were assayed for their ability to bind and displace radiolabeled GLP-1 and for their ability to stimulate the production of cAMP. The relative order of binding potency was found to be GLP-1 > exendin-4. The relative order of cyclase activation was GLP-1 = exendin-4. Affinities, as shown in the table below, differ over a 4- to 5-fold range. In contrast, *in vivo* glucose lowering potency differed over a 3430-fold range. Exendin-4 was 3430-fold more potent than GLP-1. The *in vivo* potency of exendin-4 does not match potency at the GLP-1 receptor, and is likely the culmination of an aggregate of properties.

	Binding IC50 (nM)	Cyclase EC50 (nM)	Glucose-lowering ED50 (μg)
GLP-1	0.15	0.28	20.6
Exendin-4	0.66	0.30	0.006

EXAMPLE 9 - COMPARISON OF GLYCEMIC INDICES
AND INSULIN SENSITIVITY IN PAIR-FED AND
EXENDIN-4-TREATED DIABETIC FATTY ZUCKER RATS

This Example tests whether the beneficial effects of exendin-4 in ZDF rats were secondary to changes in food intake. It compares effects obtained with exendin-4 to effects observed in saline-treated matched animals who consumed the same amount of food as was eaten by ZDF rats injected subcutaneously twice daily with 10μg exendin-4. Plasma glucose and HbA1c were measured weekly for 6 weeks.

One day after the last treatment, animals were anesthetized with halothane and subjected to an hyperinsulinemic (50 mU/kg/min) euglycemic clamp. Changes in HbA1c over 6 weeks differed between treatment groups ($P < 0.001$ ANOVA),

5 increasing in *ad lib* fed ($n=5$) and pair fed ($n=5$) rats, but decreasing in exendin-4-treated rats ($n=5$). Similarly, changes in plasma glucose differed between treatment groups ($P < 0.002$ ANOVA), increasing in *ad lib* fed and pair fed ZDF rats, and decreasing in ZDF rats treated with exendin-4. In

10 the final hour of a 3-hour clamp protocol, glucose infusion rate in exendin-4-treated rats tended to be higher than in pair fed (+105%) and *ad lib* fed (+20%) controls, respectively (10.14 ± 1.43 $n=5$, 8.46 ± 0.87 $n=4$, 4.93 ± 2.02 mg/kg/min $n=3$; n.s. $P=0.09$ ANOVA). Another index of insulin

15 sensitivity, plasma lactate concentration, differed significantly between treatment groups ($P < 0.02$ ANOVA) and was lowest in exendin-4-treated rats. Thus, exendin-4 treatment is associated with improvement in glycemic indices and in insulin sensitivity that is partly, but not fully,

20 matched in controls fed the same amount of food, indicating that improvements in metabolic control with exendin-4 in ZDF rats are at least partly due to mechanisms beyond caloric restriction.

EXAMPLE 10 - CLINICAL STUDIES AND THE STIMULATION OF
ENDOGENOUS INSULIN SECRETION BY SUBCUTANEOUS SYNTHETIC
EXENDIN-4 IN HEALTHY OVERNIGHT FASTED VOLUNTEERS

In a double blind, placebo-controlled single ascending
5 dose clinical trial to explore safety and tolerability and
pharmacokinetics of synthetic exendin-4, exendin-4
formulated for subcutaneous injection was evaluated in
healthy male volunteers while assessing effects upon plasma
glucose and insulin concentrations. Five single
10 subcutaneous doses of exendin-4 (0.01, 0.05, 0.1, 0.2 or 0.3
 $\mu\text{g/kg}$) were studied in 40 healthy male volunteers in the
fasting state. Maximum plasma exendin-4 concentrations were
achieved between 1 and 2 hours post-dose with little
difference among the doses examined. Examination of the
15 data indicated a dose dependent increase for C_{max} . There
were no serious adverse events reported in this study and in
the healthy male volunteers that participated in this study,
exendin-4 was well tolerated at subcutaneous doses up to and
including 0.1 $\mu\text{g/kg}$. A decrease in plasma glucose
20 concentration was also observed at this dose. At doses of
0.2 $\mu\text{g/kg}$ and higher, the most commonly observed adverse
events were headache, nausea, vomiting, dizziness, and
postural hypotension. There was a transient fall in plasma
glucose concentration following administration of doses of
25 0.05 $\mu\text{g/kg}$ and above.

Forty healthy, lean (mean BMI (\pm SE) 22.7 ± 1.2) subjects
aged 18-40 years were randomly assigned to 5 groups. Within
each group of 8 subjects, 6 were assigned to exendin-4 and 2
to placebo (PBO). Exendin-4 (0.01, 0.05, 0.1, 0.2 or 0.3
30 $\mu\text{g/kg}$) or placebo was administered following an overnight

fast and plasma exendin-4, glucose and insulin concentrations monitored along with safety and tolerability. No safety issues were observed. Doses ≤ 0.1 $\mu\text{g/kg}$ were tolerated as well as PBO whereas 0.2 and 0.3 $\mu\text{g/kg}$ elicited a dose-dependent increase in nausea and vomiting. Peak plasma exendin-4 concentrations rose dose-dependently and following 0.1 $\mu\text{g/kg}$, exendin-4 immunoreactivity persisted for 360 min. Plasma glucose decreased following all doses, except 0.01 $\mu\text{g/kg}$, reached a nadir by 30 min and returned back to baseline within 180 min. Subjects receiving 0.3 $\mu\text{g/kg}$ received a caloric beverage 30 minutes after dosing, precluding comparison of their data. Mean change in plasma glucose (0-180 min): 0.03 ± 0.07 , -0.07 ± 0.08 , -0.38 ± 0.14 , -0.85 ± 0.13 and -0.83 ± 0.23 mmol/L for PBO, 0.01, 0.05, 0.1, and 0.2 $\mu\text{g/kg}$ respectively; $P \leq 0.02$ versus PBO. The lowest plasma glucose recorded was 3.4 mmol/L. Corresponding mean changes in plasma insulin (0-120 min) were 0.43 ± 0.59 , 2.37 ± 0.58 , 2.28 ± 0.66 , 4.91 ± 1.23 , and 14.00 ± 3.34 $\mu\text{U/mL}$; $P \leq 0.01$ versus PBO for the 0.1 and 0.2 $\mu\text{g/kg}$ groups. Thus, in healthy, overnight fasted volunteers, subcutaneous injection of exendin-4 (1) presented no safety issues, (2) was well-tolerated at doses ≤ 0.1 $\mu\text{g/kg}$, (3) led to exendin-4 immunoreactivity in plasma for up to 6 hrs, (4) increased plasma insulin and lowered plasma glucose in a dose-dependent manner without inducing hypoglycemia.

EXAMPLE 11 - EFFECTIVENESS OF ALTERNATE
DELIVERY OF EXENDIN-4 IN RODENTS

This Example tested the delivery of exendin-4 by means alternative to injection, and examined its ability to

5 traverse mucosal surfaces in sufficient quantities to exert biological effect. Changes in concentration of plasma glucose and of intact synthetic exendin-4 (measured by a 2-site immunoradiometric assay) were observed in *db/db* mice administered a saline solution containing differing doses of

10 synthetic exendin-4 via the trachea, via an aerosol mist (pulmonary), via gavage (oral), and under the tongue (sublingual). The same routes of administration, as well as intraduodenally and nasally, were tested in rats, and bioavailability was calculated, for example, for sublingual

15 and intra-tracheal routes. Exendin-4 administered via each of the above routes in mice resulted in significant glucose-lowering activity 1 to 4 hours after administration (*db/db* mice intra-tracheal $P < 0.02$; *ob/ob* mice intra-tracheal $P < 0.0002$; *db/db* mice aerosol $P < 0.0001$; gavage $P < 0.002$; sublingual $P < 0.02$). Dose-dependent increases in plasma

20 exendin-4 concentration were up to 777 ± 365 pg/mL (*db/db* mice intra-tracheal); 170 ± 67 pg/mL (*db/db* mice aerosol); 4520 ± 1846 pg/mL (*db/db* mice sublingual). Similarly, in rats, exendin-4 concentrations were observed up to

25 $68,682 \pm 38,661$ pg/mL (intra-tracheal); 1900 pg/mL (pulmonary); 6757 pg/mL (nasal); $3,862 \pm 2,844$ pg/mL (sublingual); but no apparent absorption or biological activity when delivered intraduodenally. Bioavailability of exendin-4 in saline was ~7.3% at lower doses when delivered

30 via the trachea, where 61-74% of C_{max} was observed within 5

min. Kinetics thereafter were similar to those observed after subcutaneous administration. Bioavailability of exendin-4 in saline delivered under the tongue was 3.1-9.6% at lower doses. These studies support the delivery of
5 exendin-4 and peptide agonist analogs thereof in biologically effective quantities via convenient non-injectable routes.

10 EXAMPLE 12 - A SINGLE-BLIND, PLACEBO CONTROLLED STUDY ON
THE METABOLIC EFFECTS OF A RANGE OF DOSES OF SYNTHETIC
EXENDIN-4 GIVEN BY SUBCUTANEOUS INJECTION
TO PEOPLE WITH TYPE 2 DIABETES MELLITUS

This Example describes the results of a two-part, single-blind, placebo controlled study to examine the
15 metabolic effects of a range of doses of synthetic exendin-4 given by the subcutaneous route to subjects with Type II diabetes mellitus. The subjects involved in the study were individuals diagnosed with Type II diabetes and being controlled with diet and/or with oral hypoglycemic agents
20 (OHAs) and with HbA_{1c} concentration $\geq 7.0\%$ but $\leq 12.0\%$ at the screening visit.

The study commenced with a screening visit, after which the subjects taking OHAs were instructed to stop this medication and return to the clinic approximately 14 days
25 later when the effects of the OHA dissipated. Subjects who participated in Part 1 arrived at the clinic the afternoon prior to the first dose and began the three or four scheduled dosing days. Each dosing event was scheduled to be 24 hours apart.

Following consent and screening, subjects were randomly assigned to receive synthetic extendin-4 or placebo. In the first portion of the study, six subjects were confined to an in-patient clinical research unit for three to four days and assigned to one of 4 treatment sequences, where they were to receive each of the following doses: placebo or synthetic extendin-4 at 0.1 or 0.01, or possibly 0.001 $\mu\text{g}/\text{kg}$. Doses were administered subcutaneously following an overnight fast. A standardize liquid meal was given 15 minutes after injection of the study medication. The table below illustrates the dosing schedule for Part 1:

	Day 1	Day 2	Day 3	Day 4*
Subject 1	Placebo	0.1 $\mu\text{g}/\text{kg}$	0.01 $\mu\text{g}/\text{kg}$	0.001 $\mu\text{g}/\text{kg}$
Subject 2	Placebo	0.1 $\mu\text{g}/\text{kg}$	0.01 $\mu\text{g}/\text{kg}$	0.001 $\mu\text{g}/\text{kg}$
Subject 3	0.1 $\mu\text{g}/\text{kg}$	Placebo	0.01 $\mu\text{g}/\text{kg}$	0.001 $\mu\text{g}/\text{kg}$
Subject 4	0.1 $\mu\text{g}/\text{kg}$	Placebo	0.01 $\mu\text{g}/\text{kg}$	0.001 $\mu\text{g}/\text{kg}$
Subject 5	0.1 $\mu\text{g}/\text{kg}$	0.01 $\mu\text{g}/\text{kg}$	Placebo	0.001 $\mu\text{g}/\text{kg}$
Subject 6	0.1 $\mu\text{g}/\text{kg}$	0.01 $\mu\text{g}/\text{kg}$	Placebo	0.001 $\mu\text{g}/\text{kg}$

* Will only be completed if an effect on glucose is observed on Day 3.

In the second part of the study, approximately three days after the completion of Part 1, eight subjects were also confined to an in-patient clinical research unit for four days. The subjects were different subjects from those who participated in Part 1. The study procedures and schedule of events during Part 2 were consistent with Part 1. The doses were determined after the effect on glucose in Part 1 was analyzed.

Because there was no significant effect seen at 0.01 $\mu\text{g/kg}$ during Part 1, subjects were dosed according to the following schedule in Part 2:

	Day 1	Day 2	Day 3	Day 4
Group A	Placebo	0.02 $\mu\text{g/kg}$	0.05 $\mu\text{g/kg}$	0.1 $\mu\text{g/kg}$
Group B	0.02 $\mu\text{g/kg}$	0.1 $\mu\text{g/kg}$	Placebo	0.05 $\mu\text{g/kg}$
Group C	0.05 $\mu\text{g/kg}$	Placebo	0.1 $\mu\text{g/kg}$	0.02 $\mu\text{g/kg}$
Group D	0.1 $\mu\text{g/kg}$	0.05 $\mu\text{g/kg}$	0.02 $\mu\text{g/kg}$	Placebo

Subjects who participated in Part 2 began their dosing following review of the data from Part 1 in the same manner. All subjects returned to the clinic 4 to 6 days after discharge from the in-patient unit for a safety reassessment.

The synthetic exendin-4 used for the study was a clear colorless sterile solution for subcutaneous injection,

formulated in sodium acetate buffer (pH 4.5) and containing 4.3% mannitol as an iso-osmolality modifier. The strength of synthetic exendin-4 injection was 0.1 mg/mL. One mL of solution was supplied in 3 mL vials with rubber stoppers.

- 5 Placebo solution was made from the same sterile formulation but without the drug substance, synthetic exendin-4.

The results of the study are shown in Figures 16 and 17. They indicate the ability of various different doses of exendin-4 (0.02 $\mu\text{g/kg}$, 0.05 $\mu\text{g/kg}$, and 0.1 $\mu\text{g/kg}$) to lower
10 blood glucose in people with Type 2 diabetes.

EXAMPLE 13

This Example describes an experiment to determine a dose-response for the insulin-sensitizing effects of
15 exendin-4 and agonists thereof in Diabetic Fatty Zucker rats. The exendin-4 used in these studies was obtained from Bachem (Torrance, CA; Cat H8730, Lot 506189), American Peptides (Sunnyvale, CA; Cat 301577, Lot K1005ITI) and from
20 in-house solid-phase synthesis (lot AR1374-11; peptide content 93.3%). Thirty nine male Diabetic fatty Zucker rats (ZDF)/GmiTM-(fa/fa) (age 116 \pm 20 days; weight 441 \pm 39 g) were assigned to 5 treatment groups: saline injections only (n=9), exendin-4 injections 0.1, 1, 10 or 100 μg (n=9, 10, 6, 5, respectively). Of these, 35 rats were used in
25 hyperinsulinemic euglycemic clamp studies (n=9, 7, 9, 5, 5, respectively). Blood was sampled from the tip of the topically-anesthetized tail (Hurricane brand of 20% topical benzocaine solution, Beutlich, Waukegan, IL) of conscious overnight-fasted rats before treatment and at weekly
30 intervals for 5 weeks during treatment for analysis of

hemoglobin A_{1c} (DCA2000 latex immuno-agglutination inhibition, Bayer Diagnostics, Tarrytown, NY). Body weight was measured daily.

- After 6 weeks of treatment, ~16 hours after the last
- 5 exendin-4 (or saline) dose, and after an overnight fast, hyperinsulinemic euglycemic clamps (DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Amer J Physiol* 237:E214-23, 1979) were performed on halothane-anesthetized rats.
- 10 Rats were thermoregulated, tracheotomized and catheterized via the saphenous vein for infusion of 20% D-glucose and insulin, and via the femoral artery for blood sampling and blood pressure monitoring (P23XL transducer, Spectramed, Oxnard, CA; universal amplifier, Gould, Valley View, OH; A/D
- 15 conversion, DataTranslation, Wilmington, DE). Insulin (Humulin-R, Eli Lilly, Indianapolis, IN) was infused at 50 mU/kg/min, beginning at t=-30 min and continued until t=+180 min. Glucose was infused at a variable rate to maintain euglycemia, determined by glucose sampling and
- 20 analysis at 5 min intervals (immobilized glucose oxidase method; YSI 2300-Stat Analyzer, Yellow Springs, OH). Mean plasma glucose during clamps was 103.9 mg/dL (mean coefficient of variation was 5.8%). Glucose infusion rate data for analysis were taken from t=60-180 min when
- 25 responses had approached a steady state. Plasma lactate data, obtained from an immobilized lactate oxidase sensor incorporated in the glucose analyzer, were also collected.
- Injections were given intraperitoneally at ~8 a.m. and 4 p.m., Monday through Friday, and at ~10 a.m. on Saturday
- 30 and Sunday.

Pairwise statistical analyses were performed using Student's t-test routines (Instat v3.0, GraphPad Software, San Diego, CA) using $P < 0.05$ as the level of significance. Dose-response analyses used 4-parameter logistic regression and general effects were tested using one-way ANOVA (Prism v3.0, GraphPad Software, San Diego, CA).

The results showed that in Diabetic Fatty Zucker rats treated with different doses of exendin-4 for 6 weeks, there was a dose-dependent reduction in food intake (ED_{50} $0.14 \mu\text{g} \pm 0.15$ log; see Fig 13a), and in body weight (ED_{50} $0.42 \mu\text{g} \pm 0.15$ log; see Fig 13b) of up to 27 ± 2 g, representing a $5.6 \pm 0.5\%$ decrease in body weight relative to saline-injected controls.

In this group of rats, the diabetic course appeared progressive, since hemoglobin A_{1c} initially rose in all groups. Injection of exendin-4 nonetheless appeared to dose-dependently arrest and reverse the rise in hemoglobin A_{1c} (see Fig 13c). The exendin-4 dose-response for effect on hemoglobin A_{1c} measured during the last 2 weeks of treatment was generally significant ($P = 0.05$ ANOVA) and specifically at $1 \mu\text{g}$ and $100 \mu\text{g}$ doses ($P < 0.005$, $P < 0.02$ respectively). A similar pattern was observed in relation to fasting plasma triglycerides in the last 2 weeks of treatment, where plasma concentrations were significantly reduced at all doses by between 51% and 65% ($P < 0.002$ ANOVA).

Thirty five of the 39 rats entered into the study progressed to an hyperinsulinemic, euglycemic clamp ~16 hours after their last treatment. Initial fasting plasma glucose concentrations, higher in saline-treated ($489 \pm 28 \text{ mg/dL}$) than exendin-treated rats, fell with insulin

infusion and were subsequently clamped at similar plasma glucose concentrations (105.6 mg/dL at 60-180 min; mean coefficient of variation 4.6%; see Fig 14a). Glucose infusion rate required to maintain euglycemia was dose-dependently increased by prior treatment with exendin-4 (ED50 $1.0\mu\text{g} \pm 0.41 \log$; see Fig 14b). Exendin-4 treatment increased glucose infusion rate by up to 48% relative to saline-treated controls.

Plasma lactate concentration before and during the clamp procedure was dose-dependently reduced by prior treatment with exendin-4 (ED50 $4\mu\text{g} \pm 0.25 \log$; see Fig 14c). This effect, representing up to a 42% reduction in mean plasma lactate concentration between 60 and 180 minutes of the clamp, appeared primarily due to a reduction in pre-clamp (basal) lactate concentration; increments in plasma lactate during hyperinsulinemia were similar in all treatment groups. There were no treatment-related differences in mean arterial pressure measured before or during clamp procedures.

The approximately 50% increase in insulin sensitivity observed after chronic administration of exendin-4 was both important and surprising in view of observations that exendin-4 has no acute effect in insulin-sensitive tissues *in vitro* (i.e. no effect on basal or insulin-stimulated incorporation of radiolabeled glucose into glycogen in isolated soleus muscle, or into lipid in isolated adipocytes; Pittner et al., unpublished). Although the possibility that the increase in insulin sensitivity may have resulted in some part from improved glycemic control and reduced glucose toxicity may not be overlooked, it has

- been reported that the increase in insulin sensitivity from various antidiabetic therapies, including those not classed as insulin sensitizing, is quite variable and it has been reported that acute treatment with GLP-1 appears not to
- 5 immediately alter insulin sensitivity in humans (Orskov L, Holst JJ, Moller J, Orskov C, Moller N, Alberti KG, Schmitz O: GLP-1 does not not acutely affect insulin sensitivity in healthy man. *Diabetologia* 39:1227-32, 1996; Ahren B, Larsson H, Holst JJ: Effects of glucagon-like peptide-1 on
- 10 islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:473-8, 1997; UK Prospective Diabetes Study Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of
- 15 complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837-53, 1998). Thus chronic administration of exendin-4 appears to be associated with increases in insulin sensitivity that are as great as, if not greater than, those observed with other therapies, including insulin sensitizing
- 20 drugs such as thiazolidinediones and metformin.

EXAMPLE 14

Preparation of amidated peptide having SEQ. ID. NO. 9

- The above-identified peptide was assembled on 4-(2'-4'-
- 25 dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was
- 30 employed. However, at some positions coupling was less

efficient than expected and double couplings were required. In particular, residues Asp₉, Thr₇, and Phe₆ all required double coupling. Deprotection (Fmoc group removal) of the growing peptide chain using piperidine was not always
5 efficient. Double deprotection was required at positions Arg₂₀, Val₁₉, and Leu₁₄. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to
10 standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity
15 was about 55%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B
20 in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide
25 gave product peptide having an observed retention time of 14.5 minutes. Electrospray Mass Spectrometry (M): calculated 4131.7; found 4129.3.

EXAMPLE 15Preparation of Peptide having SEQ. ID. NO. 10

- The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 10 Analytical RP-HPLC (gradient 25% to 75% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 21.5 minutes. Electrospray Mass Spectrometry (M): calculated 4168.6; found 4171.2.

15

EXAMPLE 16Preparation of Peptide having SEQ. ID. NO. 11

- The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 4147.6; found 4150.2.

EXAMPLE 17Preparation of Peptide having SEQ. ID. NO. 12

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 35% to 65% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.7 minutes. Electrospray Mass Spectrometry (M): calculated 4212.6; found 4213.2.

15

EXAMPLE 18Preparation of Peptide having SEQ. ID. NO. 13

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
25 Analytical RP-HPLC (gradient 30% to 50% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 16.3 minutes. Electrospray Mass Spectrometry (M): calculated 4262.7; found 4262.4.

30

EXAMPLE 19

Preparation of Peptide having SEQ. ID. NO. 14

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
- 5 Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
- 10 A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6

EXAMPLE 20Preparation of Peptide having SEQ. ID. NO. 15

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4224.7.

15

EXAMPLE 21Preparation of Peptide having SEQ. ID. NO. 16

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6

30

EXAMPLE 22Preparation of Peptide having SEQ. ID. NO. 17

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 14. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4186.6.

15

EXAMPLE 23Preparation of Peptide having SEQ. ID. NO. 18

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 14. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4200.7.

30

EXAMPLE 24Preparation of Peptide having SEQ. ID. NO. 19

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4200.7.

15

EXAMPLE 25Preparation of Peptide having SEQ. ID. NO. 20

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4202.7.

30

EXAMPLE 26Preparation of Peptide having SEQ. ID. NO. 21

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4145.6.

15

EXAMPLE 27Preparation of Peptide having SEQ. ID. NO. 22

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4184.6.

30

EXAMPLE 28Preparation of Peptide having SEQ. ID. NO. 23

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4145.6.

15

EXAMPLE 29Preparation of Peptide having SEQ. ID. NO. 24

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4224.7.

30

EXAMPLE 30Preparation of Peptide having SEQ. ID. NO. 25

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6.

15

EXAMPLE 31Preparation of Peptide having SEQ. ID. NO. 26

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4115.5.

30

EXAMPLE 32Preparation of Peptide having SEQ. ID. NO. 27

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4188.6.

15

EXAMPLE 33Preparation of Peptide having SEQ. ID. NO. 28

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
- 25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4131.6.

30

EXAMPLE 34Preparation of Peptide having SEQ. ID. NO. 29

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 14. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
10 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4172.6.

15

EXAMPLE 35Preparation of Peptide having SEQ. ID. NO. 30

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 14. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4145.6.

30

EXAMPLE 36Preparation of Peptide having SEQ. ID. NO. 31

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are required at the thioproline positions 38, 37, 36 and 31.
- 10 Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass
- 15 Spectrometry (M): calculated 4266.8.

EXAMPLE 37Preparation of Peptide having SEQ. ID. NO. 32

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
- 20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are required at the thioproline positions 38, 37 and 36. Used in
- 25 analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time

of the product peptide. Electrospray Mass Spectrometry (M): calculated 4246.8.

EXAMPLE 38

Preparation of Peptide having SEQ. ID. NO. 33

- 5 The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 10 similar way to Example 14. Additional double couplings are required at the homoproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
- 15 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4250.8.

EXAMPLE 39

Preparation of Peptide having SEQ. ID. NO. 34

- 20 The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 25 cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are required at the homoproline positions 38, 37, and 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
- 30 Solvent B in Solvent A over 30 minutes) of the lyophilized

peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4234.8.

5

EXAMPLE 40Preparation of Peptide having SEQ. ID. NO. 35

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are required at the thioproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4209.8.

20

EXAMPLE 41Preparation of Peptide having SEQ. ID. NO. 36

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are required at the homoproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and

30

Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4193.7.

EXAMPLE 42

Preparation of Peptide having SEQ. ID. NO. 37

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are required at the N-methylalanine positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3858.2.

EXAMPLE 43

Preparation of Peptide having SEQ. ID. NO. 38

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are

required at the N-methylalanine positions 38, 37 and 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3940.3.

EXAMPLE 44

10 Preparation of Peptide having SEQ. ID. NO. 39

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
15 cleaved from the resin, deprotected and purified in a similar way to Example 14. Additional double couplings are required at the N-methylalanine positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient
20 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3801.1.

25 EXAMPLE 45

Preparation of C-terminal carboxylic acid Peptides corresponding to the above C-terminal amide sequences.

The above peptides of Examples 1 to 30 are assembled on the so called Wang resin (p-alkoxybenzylalcohol resin
30 (Bachem, 0.54 mmole/g)) using Fmoc-protected amino acids

(Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry provides an experimentally determined (M).

EXAMPLE 46

Preparation of Peptide having SEQ ID NO. 7

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-
NH₂ [SEQ. ID. NO. 7]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. Deprotection (Fmoc group removal) of the growing peptide chain was achieved using piperidine. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in

glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 75%.

- Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 50% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 18.9 minutes. Electrospray Mass Spectrometry (M): calculated 3408.0; found 3408.9.

EXAMPLE 47

Preparation of Peptide having SEQ ID NO. 40

- His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
20 Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 40]

- The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
25 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
30 Analytical RP-HPLC (gradient 30% to 40% Solvent B in Solvent

A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 3294.7; found 3294.8.

5

EXAMPLE 48Preparation of Peptide having SEQ ID NO. 41

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
10 ID. NO. 41]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
15 Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
20 Analytical RP-HPLC (gradient 29% to 36% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 20.7 minutes. Electrospray Mass Spectrometry (M): calculated 3237.6; found 3240.

25

EXAMPLE 49Preparation of Peptide having SEQ ID NO. 42

His Ala Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
30 ID. NO. 42]

- The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 5 cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product
- 10 peptide having an observed retention time of 15.2 minutes. Electrospray Mass Spectrometry (M): calculated 3251.6; found 3251.5.

EXAMPLE 50

- 15 Preparation of Peptide having SEQ ID NO. 43

His Gly Glu Gly Ala Phe Thr Ser Asp Leu Ser Lys Gln Leu
Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂
[SEQ. ID. NO. 43]

- 20 The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 25 cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 13.1 minutes.

Electrospray Mass Spectrometry (M): calculated 3207.6; found 3208.3.

EXAMPLE 51

5 Preparation of Peptide having SEQ ID NO. 44

His Gly Glu Gly Thr Ala Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 44]

- 10 The above amidated peptide was assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
15 similar way to Example 46. Used in analysis were Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 35% to 45% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 12.8 minutes.
20 Electrospray Mass Spectrometry (M): calculated 3161.5; found
3163.

EXAMPLE 52

25 Preparation of Peptide having SEQ ID NO. 45

- His Gly Glu Gly Thr Phe Thr Ala Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 45]

- 30 The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using

Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis were Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

- 5 Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 15.2 minutes.
Electrospray Mass Spectrometry (M): calculated 3221.6; found
3222.7.

10

EXAMPLE 53

Preparation of Peptide having SEQ ID NO. 46

- His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
15 ID. NO. 46]

- The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis were Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 34% to 44% Solvent B in Solvent
25 A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 14.3 minutes.
Electrospray Mass Spectrometry (M): calculated 3195.5; found
3199.4.

EXAMPLE 54Preparation of Peptide having SEQ ID NO. 47

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
5 ID. NO. 47]

- The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis were Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 15.7 minutes.
Electrospray Mass Spectrometry (M): calculated 3221.6; found
3221.6.

20

EXAMPLE 55Preparation of Peptide having SEQ ID NO. 48

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 48]

25

- The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

- similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product
- 5 peptide having an observed retention time of 18.1 minutes. Electrospray Mass Spectrometry (M): calculated 3180.5; found 3180.9.

EXAMPLE 56

10 Preparation of Peptide having SEQ ID NO. 49

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Ala Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 49]

- 15 The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.0 minutes.
- 25 Electrospray Mass Spectrometry (M): calculated 3180.6; found 3182.8.

EXAMPLE 57Preparation of Peptide having SEQ ID NO. 50

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 50]

The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis were Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 32% to 42% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 14.9 minutes.
Electrospray Mass Spectrometry (M): calculated 3195.5; found
3195.9.

20

EXAMPLE 58Preparation of Peptide having SEQ ID NO. 51

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Ala
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 51]

25

The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product
5 peptide having an observed retention time of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3179.0.

EXAMPLE 59

10 Preparation of Peptide having SEQ ID NO. 52

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Ala Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 52]

15 The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 14.3 minutes.
25 Electrospray Mass Spectrometry (M): calculated 3179.6; found 3180.0.

EXAMPLE 60Preparation of Peptide having SEQ ID NO. 53

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Ala Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
5 ID. NO. 53]

- The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
- 10 Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47% Solvent B in Solvent
- 15 A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 13.7 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3179.0.

20

EXAMPLE 61Preparation of Peptide having SEQ ID NO. 54

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Ala Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 54]

- 25 The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 30 similar way to Example 46. Used in analysis were Solvent A

(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

Analytical RP-HPLC (gradient 35% to 45% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.0 minutes.

- 5 Electrospray Mass Spectrometry (M): calculated 3209.6; found 3212.8.

EXAMPLE 62

Preparation of Peptide having SEQ ID NO. 55

- 10 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Ala Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 55]

- The above-identified amidated peptide was assembled on
15 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis were Solvent A
20 (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 14.3 minutes.
Electrospray Mass Spectrometry (M): calculated 3152.5; found
25 3153.5.

EXAMPLE 63Preparation of Peptide having SEQ ID NO. 56

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Ala Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 56]

- The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
- 10 Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35% to 45% Solvent B in Solvent
- 15 A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 12.1 minutes. Electrospray Mass Spectrometry (M): calculated 3195.5; found 3197.7.

20

EXAMPLE 64Preparation of Peptide having SEQ ID NO. 57

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Ala Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 57]

25

- The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 30 cleaved from the resin, deprotected and purified in a

110

- similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product
- 5 peptide having an observed retention time of 10.9 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3180.5.

EXAMPLE 65

10 Preparation of Peptide having SEQ ID NO. 58

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn-NH₂ [SEQ.
ID. NO. 58]

- 15 The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 32% to 42% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.5 minutes.
- 25 Electrospray Mass Spectrometry (M): calculated 3161.5; found 3163.0.

111

EXAMPLE 66Preparation of Peptide having SEQ ID NO. 59

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Ala Lys Asn-NH₂ [SEQ.
5 ID. NO. 59]

The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis were Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 32% to 42% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 19.5 minutes.
Electrospray Mass Spectrometry (M): calculated 3195.5; found
3199.

20

EXAMPLE 67Preparation of Peptide having SEQ ID NO. 60

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Ala Asn-NH₂ [SEQ.
ID. NO. 60]

25

The above-identified amidated peptide was assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

112

- similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product
- 5 peptide having an observed retention time of 14.5 minutes. Electrospray Mass Spectrometry (M): calculated 3180.5; found 3183.7.

EXAMPLE 68

10 Preparation of Peptide having SEQ ID NO. 61

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Ala-NH₂ [SEQ.
ID. NO. 61]

- 15 The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 34% to 44% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 22.8 minutes.
- 25 Electrospray Mass Spectrometry (M): calculated 3194.6; found 3197.6.

EXAMPLE 69Preparation of Peptide having SEQ ID NO. 62

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 Pro Ser Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 62]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4099.6.

20

EXAMPLE 70Preparation of Peptide having SEQ ID NO. 63

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 63]
25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

- similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4042.5.

EXAMPLE 71

10 Preparation of Peptide having SEQ ID NO. 64

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 64]

- 15 The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4002.4.

EXAMPLE 72Preparation of Peptide having SEQ ID NO. 65

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
5 Pro Ser Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 65]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3945.4.

20

EXAMPLE 73Preparation of Peptide having SEQ ID NO. 66

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 66]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

116

similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3905.3.

EXAMPLE 74

10 Preparation of Peptide having SEQ ID NO. 67

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 67]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3848.2.

EXAMPLE 75Preparation of Peptide having SEQ ID NO. 68

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 Pro Ser Ser Gly Ala-NH₂ [SEQ. ID. NO. 68]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3808.2.

20

EXAMPLE 76Preparation of Peptide having SEQ ID NO. 69

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala-NH₂ [SEQ. ID. NO. 69]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

- similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3751.1.

EXAMPLE 77

10 Preparation of Peptide having SEQ ID NO. 70

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly-NH₂ [SEQ. ID. NO. 70]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 20 cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3737.1.

EXAMPLE 78Preparation of Peptide having SEQ ID NO. 71

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
5 Pro Ser Ser Gly-NH₂ [SEQ. ID. NO. 71]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3680.1.

20

EXAMPLE 79Preparation of Peptide having SEQ ID NO. 72

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser-NH₂ [SEQ. ID. NO. 72]
25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

120

similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3680.1

EXAMPLE 80

10 Preparation of Peptide having SEQ ID NO. 73

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser-NH₂ [SEQ. ID. NO. 73]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3623.0.

EXAMPLE 81Preparation of Peptide having SEQ ID NO. 74

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly

5 Pro Ser-NH₂ [SEQ. ID. NO. 74]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3593.0

20

EXAMPLE 82Preparation of Peptide having SEQ ID NO. 75

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser-NH₂ [SEQ. ID. NO. 75]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3535.9

EXAMPLE 83

10 Preparation of Peptide having SEQ ID NO. 76

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro-NH₂ [SEQ. ID. NO. 76]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3505.94.

EXAMPLE 84Preparation of Peptide having SEQ ID NO. 77

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
5 Pro-NH₂ [SEQ. ID. NO. 77]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3448.8.

20

EXAMPLE 85Preparation of Peptide having SEQ ID NO. 78

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly-
25 NH₂ [SEQ. ID. NO. 78]

25

The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

124

similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3351.7.

EXAMPLE 86

10 Preparation of Peptide having SEQ ID NO. 79

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly-NH₂
[SEQ. ID. NO. 79]

- 15 The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3351.8.

EXAMPLE 87Preparation of Peptide having SEQ ID NO. 80

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly-NH₂

5 [SEQ. ID. NO. 80]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3294.7.

20

EXAMPLE 88Preparation of Peptide having SEQ ID NO. 81

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
tPro Ser Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 81]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

126

similar way to Example 46. Double couplings are required at residues 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4197.1.

EXAMPLE 89

10 Preparation of Peptide having SEQ ID NO. 82

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 82]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 46. Double couplings are required at residues 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4179.1.

EXAMPLE 90Preparation of Peptide having SEQ ID NO. 83

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 NMeala Ser Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 83]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Double couplings are required at
residues 36 and 31. Used in analysis are Solvent A (0.1%
TFA in water) and Solvent B (0.1% TFA in ACN). Analytical
15 RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out to
determine the retention time of the product peptide.
Electrospray Mass Spectrometry (M): calculated 3948.3.

20

EXAMPLE 91Preparation of Peptide having SEQ ID NO. 84

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
NMeala Ser Ser Gly Ala NMeala Nmeala-NH₂ [SEQ. ID. NO. 84]
25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 46. Double couplings are required at residues 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3840.1.

EXAMPLE 92

10 Preparation of Peptide having SEQ ID NO. 85

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
hPro Ser Ser Gly Ala hPro hPro-NH₂ [SEQ. ID. NO. 85]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 46. Double couplings are required at residues 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to
25 determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4050.1.

EXAMPLE 93Preparation of Peptide having SEQ ID NO. 86

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 hPro Ser Ser Gly Ala hPro-NH₂ [SEQ. ID. NO. 86]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. A double coupling is required at
residue 31. Used in analysis are Solvent A (0.1% TFA in
water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
15 (gradient 30% to 60% Solvent B in Solvent A over 30 minutes)
of the lyophilized peptide is then carried out to determine
the retention time of the product peptide. Electrospray
Mass Spectrometry (M): calculated 3937.1.

20

EXAMPLE 94Preparation of Peptide having SEQ ID NO. 87

Arg Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala-NH₂ [SEQ. ID. NO. 87]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3827.2.

EXAMPLE 95

10 Preparation of Peptide having SEQ ID NO. 88

His Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-
NH₂ [SEQ. ID. NO. 88]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3394.8.

EXAMPLE 96Preparation of Peptide having SEQ ID NO. 89

His Gly Glu Gly Thr Naphthylala Thr Ser Asp Leu Ser Lys Gln
Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-
5 NH₂ [SEQ. ID. NO. 89]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3289.5.

20

EXAMPLE 97Preparation of Peptide having SEQ ID NO. 90

His Gly Glu Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 90]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

- similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3280.7.

EXAMPLE 98

10 Preparation of Peptide having SEQ ID NO. 91

His Gly Glu Gly Thr Phe Ser Thr Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 91]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3294.7.

EXAMPLE 99Preparation of Peptide having SEQ ID NO. 92

His Gly Glu Gly Thr Phe Ser Glu Leu Ser Lys Gln Met Ala
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 92]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3250.7.

20

EXAMPLE 100Preparation of Peptide having SEQ ID NO. 93

His Gly Glu Gly Thr Phe Thr Ser Asp pentylgly Ser Lys Gln
Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-
NH₂ [SEQ. ID. NO. 93]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
20 cleaved from the resin, deprotected and purified in a

134

- similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3253.5.

EXAMPLE 101

10 Preparation of Peptide having SEQ ID NO. 94

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Naphthylala Ile Glu Phe Leu Lys Asn-
NH₂ [SEQ. ID. NO. 94]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product
- 25 peptide. Electrospray Mass Spectrometry (M): calculated 3289.5.

EXAMPLE 102Preparation of Peptide having SEQ ID NO. 95

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe tButylgly Glu Trp Leu Lys Asn-NH₂

5 [SEQ. ID. NO. 95]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3183.4.

20

EXAMPLE 103Preparation of Peptide having SEQ ID NO. 96

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Asp Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 96]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

136

similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then

5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3237.6.

EXAMPLE 104

10 Preparation of Peptide having SEQ ID NO. 97

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser-NH₂ [SEQ. ID. NO. 97]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a

20 similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product

25 peptide. Electrospray Mass Spectrometry (M): calculated 3637.9.

EXAMPLE 105Preparation of Peptide having SEQ ID NO. 98

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly-NH₂

5 [SEQ. ID. NO. 98]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3309.7.

20

EXAMPLE 106Preparation of Peptide having SEQ ID NO. 99

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
hPro Ser Ser Gly Ala hPro hPro-NH₂ [SEQ. ID. NO. 99]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

138

similar way to Example 46. Double couplings are required at residues 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3711.1.

EXAMPLE 107

10 Preparation of C-terminal carboxylic acid peptides
 corresponding to the above C-terminal amide sequences for
 SEQ ID NOS. 7, 40-61, 68-75, 78-80 and 87-98

 Peptides having the sequences of SEQ ID NOS. 7, 40-61, 68-75, 78-80 and 87-98 are assembled on the so called Wang resin (p-alkoxybenzylalcohol resin (Bachem, 0.54 mmole/g))
15 using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
20 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry provides an experimentally determined (M).

25

EXAMPLE 108

Preparation of C-terminal carboxylic acid peptides
corresponding to the above C-terminal amide sequences for
SEQ ID NOS. 62-67, 76, 77, 81-86 and 99

- 5 Peptides having the sequences of SEQ ID NOS. 62-67, 76,
77, 81-86 and 99 are assembled on the 2-chlorotritylchloride
resin (200-400 mesh), 2% DVB (Novabiochem, 0.4-1.0 mmole/g))
using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
10 similar way to Example 46. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
15 peptide. Electrospray Mass Spectrometry provides an
experimentally determined (M).

EXAMPLE 109

Preparation of Peptide having SEQ ID NO. 100

- 20 Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 100]

- The above amidated peptide was assembled on 4-(2'-4'-
25 dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.). In
general, single-coupling cycles were used throughout the
synthesis and Fast Moc (HBTU activation) chemistry was
30 employed. Deprotection (Fmoc group removal) of the growing

peptide chain was achieved using piperidine. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and

5 trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized

10 peptide was dissolved in water). Crude purity was about 75%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a

15 preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B

20 in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.2 minutes. Electrospray Mass Spectrometry (M): calculated 3171.6; found 3172.

25

EXAMPLE 110

Preparation of Peptide having SEQ ID NO. 101

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 101]

30

141

- The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 5 cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product
- 10 peptide having an observed retention time of 14.9 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3180.

EXAMPLE 111

- 15 Preparation of Peptide having SEQ ID NO. 102

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 102]

- 20 The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 25 cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 12.2 minutes.

Electrospray Mass Spectrometry (M): calculated 3251.6; found 3253.3.

EXAMPLE 112

5 Preparation of Peptide having SEQ ID NO. 103

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 103]

- 10 The above amidated peptide was assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
15 similar way to Example 109. Used in analysis were Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 35% to 45% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide gave product
peptide having an observed retention time of 16.3 minutes.
20 Electrospray Mass Spectrometry (M): calculated 3193.6; found
3197.

EXAMPLE 113

Preparation of Peptide having SEQ ID NO. 104

- 25 Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 104]

- 30 The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3228.6.

EXAMPLE 114

Preparation of Peptide having SEQ ID NO. 105

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
15 Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 105]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
25 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3234.7.
30

EXAMPLE 115Preparation of Peptide having SEQ ID NO. 106

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂

5 [SEQ. ID. NO. 106]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3308.7.

20

EXAMPLE 116Preparation of Peptide having SEQ ID NO. 107

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 107]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3250.7

EXAMPLE 117

10 Preparation of Peptide having SEQ ID NO. 108

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 108]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3252.6.

EXAMPLE 118Preparation of Peptide having SEQ ID NO. 109

Ala Ala Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 109]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3200.6.

20

EXAMPLE 119Preparation of Peptide having SEQ ID NO. 110

Ala Ala Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 110]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3143.5.

EXAMPLE 120

10 Preparation of Peptide having SEQ ID NO. 111

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 111]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3214.6.

EXAMPLE 121Preparation of Peptide having SEQ ID NO. 112

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 112]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3157.5.

20

EXAMPLE 122Preparation of Peptide having SEQ ID NO. 113

Ala Gly Asp Gly Ala Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 113]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

149

- similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3184.6.

EXAMPLE 123

10 Preparation of Peptide having SEQ ID NO. 114

Ala Gly Asp Gly Ala Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 114]

- The above-identified amidated peptide is assembled on
- 15 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A
- 20 (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated
- 25 3127.5.

EXAMPLE 124Preparation of Peptide having SEQ ID NO. 115

Ala Gly Asp Gly Thr NaphthylAla Thr Ser Asp Leu Ser Lys Gln
Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-

5 NH_2 [SEQ. ID. NO. 115]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3266.4.

20

EXAMPLE 125Preparation of Peptide having SEQ ID NO. 116

Ala Gly Asp Gly Thr NaphthylAla Thr Ser Asp Leu Ser Lys Gln
Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-
25 NH_2 [SEQ. ID. NO. 116]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3209.4.

EXAMPLE 126

10 Preparation of Peptide having SEQ ID NO. 117

Ala Gly Asp Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 117]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3200.6.

EXAMPLE 127Preparation of Peptide having SEQ ID NO. 118

Ala Gly Asp Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 118]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using

10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3143.5.

20

EXAMPLE 128Preparation of Peptide having SEQ ID NO. 119

Ala Gly Asp Gly Thr Phe Thr Ala Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 119]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

153

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3198.6.

EXAMPLE 12910 Preparation of Peptide having SEQ ID NO. 120

Ala Gly Asp Gly Thr Phe Thr Ala Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 120]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3141.5.

EXAMPLE 130Preparation of Peptide having SEQ ID NO. 121

Ala Gly Asp Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
5 ID. NO. 121]

- The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
- 10 Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
- 15 A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3170.6.

20

EXAMPLE 131Preparation of Peptide having SEQ ID NO. 122

Ala Gly Asp Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 122]

25

- The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
- 30 cleaved from the resin, deprotected and purified in a

155

- similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3113.5.

EXAMPLE 132

10 Preparation of Peptide having SEQ ID NO. 123

Ala Gly Asp Gly Thr Phe Thr Ser Glu Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 123]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3228.6.

EXAMPLE 133Preparation of Peptide having SEQ ID NO. 124

Ala Gly Asp Gly Thr Phe Thr Ser Glu Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 124]

- The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3171.6.

20

EXAMPLE 134Preparation of Peptide having SEQ ID NO. 125

Ala Gly Asp Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 125]

25

- The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

157

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3172.5.

EXAMPLE 135

10 Preparation of Peptide having SEQ ID NO. 126

Ala Gly Asp Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 126]

15 The above-identified amidated peptiden is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3115.4.

EXAMPLE 136Preparation of Peptide having SEQ ID NO. 127

Ala Gly Asp Gly Thr Phe Thr Ser Asp Pentylgly Ser Lys Gln
Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-

5 NH₂ [SEQ. ID. NO. 127]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3230.4.

20

EXAMPLE 137Preparation of Peptide having SEQ ID NO. 128

Ala Gly Asp Gly Thr Phe Thr Ser Asp Pentylgly Ser Lys Gln
Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-
25 NH₂ [SEQ. ID. NO. 128]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

159

- similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3198.6.

EXAMPLE 138

10 Preparation of Peptide having SEQ ID NO. 129

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 129]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3141.5.

EXAMPLE 139Preparation of Peptide having SEQ ID NO. 130

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 130]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3157.5.

20

EXAMPLE 140Preparation of Peptide having SEQ ID NO. 131

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 131]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

161

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3100.4.

EXAMPLE 14110 Preparation of Peptide having SEQ ID NO. 132

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 132]

The above-identified amidated peptide is assembled on
15 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A
20 (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated
25 3157.6.

EXAMPLE 142Preparation of Peptide having SEQ ID NO. 133

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Ala Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 133]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using

10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3100.5.

20

EXAMPLE 143Preparation of Peptide having SEQ ID NO. 134

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Ala Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 134]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

163

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3100.5.

EXAMPLE 14410 Preparation of Peptide having SEQ ID NO. 135

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 135]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3154.5.

EXAMPLE 145Preparation of Peptide having SEQ ID NO. 136

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
5 ID. NO. 136]

- The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3115.5.

20

EXAMPLE 146Preparation of Peptide having SEQ ID NO. 137

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln
Pentylgly Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu
Lys Asn-NH₂ [SEQ. ID. NO. 137]

25

- The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

165

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3212.4.

EXAMPLE 14710 Preparation of Peptide having SEQ ID NO. 138

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln
Pentylgly Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu
Lys Asn-NH₂ [SEQ. ID. NO. 138]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3173.4.

EXAMPLE 148Preparation of Peptide having SEQ ID NO. 139

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Ala
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
5 ID. NO. 139]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3156.6.

20

EXAMPLE 149Preparation of Peptide having SEQ ID NO. 140

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Ala
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 140]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

167

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3099.5.

EXAMPLE 15010 Preparation of Peptide having SEQ ID NO. 141

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Ala Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 141]

The above-identified amidated peptide is assembled on
15 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
20 (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
25 3156.6.

EXAMPLE 151Preparation of Peptide having SEQ ID NO. 142

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Ala Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
5 ID. NO. 142]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3099.5.

20

EXAMPLE 152Preparation of Peptide having SEQ ID NO. 143

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Ala Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 143]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3156.6.

EXAMPLE 153

10 Preparation of Peptide having SEQ ID NO. 144

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Ala Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 144]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3099.5.

EXAMPLE 154Preparation of Peptide having SEQ ID NO. 145

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Ala Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 145]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3186.6.

20

EXAMPLE 155Preparation of Peptide having SEQ ID NO. 146

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Ala Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 146]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

171

- similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3129.5.

EXAMPLE 156

10 Preparation of Peptide having SEQ ID NO. 147

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Ala Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 147]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3129.5.

EXAMPLE 157Preparation of Peptide having SEQ ID NO. 148

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Ala Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 148]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3072.4.

20

EXAMPLE 158Preparation of Peptide having SEQ ID NO. 149

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Ala Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 149]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3172.5.

EXAMPLE 159

10 Preparation of Peptide having SEQ ID NO. 150

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Ala Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 150]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3115.5.

EXAMPLE 160Preparation of Peptide having SEQ ID NO. 151

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Naphthylala Ile Glu Trp Leu Lys Asn-
5 NH₂ [SEQ. ID. NO. 151]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3266.4.

20

EXAMPLE 161Preparation of Peptide having SEQ ID NO. 152

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Naphthylala Ile Glu Phe Leu Lys Asn-
NH₂ [SEQ. ID. NO. 152]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

175

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3209.4.

EXAMPLE 162

10 Preparation of Peptide having SEQ ID NO. 153

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Val Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 153]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3200.6.

EXAMPLE 163Preparation of Peptide having SEQ ID NO. 154

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Val Glu Phe Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 154]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3143.5.

20

EXAMPLE 164Preparation of Peptide having SEQ ID NO. 155

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe tButylgly Glu Trp Leu Lys Asn-NH₂
[SEQ. ID. NO. 155]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

177

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3216.5.

EXAMPLE 165

10 Preparation of Peptide having SEQ ID NO. 156

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe tButylgly Glu Phe Leu Lys Asn-NH₂
[SEQ. ID. NO. 156]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3159.4.

EXAMPLE 166Preparation of Peptide having SEQ ID NO. 157

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Asp Trp Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 157]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3200.6.

20

EXAMPLE 167Preparation of Peptide having SEQ ID NO. 158

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Asp Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 158]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3143.5.

EXAMPLE 168

10 Preparation of Peptide having SEQ ID NO. 159

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn-NH₂ [SEQ.
ID. NO. 159]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3099.5.

EXAMPLE 169Preparation of Peptide having SEQ ID NO. 160

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn-NH₂ [SEQ.

5 ID. NO. 160]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3081.4.

20

EXAMPLE 170Preparation of Peptide having SEQ ID NO. 161

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Ala Lys Asn-NH₂ [SEQ.
ID. NO. 161]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3172.5.

EXAMPLE 171

10 Preparation of Peptide having SEQ ID NO. 162

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Ala Lys Asn-NH₂ [SEQ.
ID. NO. 162]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3115.5.

EXAMPLE 172Preparation of Peptide having SEQ ID NO. 163

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Ala Asn-NH₂ [SEQ.

5 ID. NO. 163]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3157.5.

20

EXAMPLE 173Preparation of Peptide having SEQ ID NO. 164

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Ala Asn-NH₂ [SEQ.
ID. NO. 164]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

- similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3100.4.

EXAMPLE 174

10 Preparation of Peptide having SEQ ID NO. 165

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Ala-NH₂ [SEQ.
ID. NO. 165]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3171.6.

EXAMPLE 175Preparation of Peptide having SEQ ID NO. 166

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Ala-NH₂ [SEQ.

5 ID. NO. 166]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3114.5.

20

EXAMPLE 176Preparation of Peptide having SEQ ID NO. 167

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 167]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4033.5.

EXAMPLE 177

10 Preparation of Peptide having SEQ ID NO. 168

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 168]

The above-identified amidated peptide is assembled on
15 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A
20 (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated
25 3984.4.

EXAMPLE 178Preparation of Peptide having SEQ ID NO. 169

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 Pro Ser Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 169]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4016.5.

20

EXAMPLE 179Preparation of Peptide having SEQ ID NO. 170

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
25 Pro Ser Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 170]

30

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3861.3.

EXAMPLE 180

10 Preparation of Peptide having SEQ ID NO. 171

Ala Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 171]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3746.1.

EXAMPLE 181Preparation of Peptide having SEQ ID NO. 172

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 Pro Ser Ser Gly Ala-NH₂ [SEQ. ID. NO. 172]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3742.1.

20

EXAMPLE 182Preparation of Peptide having SEQ ID NO. 173

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala-NH₂ [SEQ. ID. NO. 173]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3693.1.

EXAMPLE 183

10 Preparation of Peptide having SEQ ID NO. 174

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly-NH₂ [SEQ. ID. NO. 174]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3751.2.

190

EXAMPLE 184Preparation of Peptide having SEQ ID NO. 175

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 Pro Ser Ser-NH₂ [SEQ. ID. NO. 175]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3634.1.

20

EXAMPLE 185Preparation of Peptide having SEQ ID NO. 176

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser-NH₂ [SEQ. ID. NO. 176]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3526.9.

EXAMPLE 186

10 Preparation of Peptide having SEQ ID NO. 177

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser-NH₂ [SEQ. ID. NO. 177]

The above-identified amidated peptide is assembled on
15 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
20 (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
25 3477.9.

EXAMPLE 187Preparation of Peptide having SEQ ID NO. 178

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly

5 Pro-NH₂ [SEQ. ID. NO. 178]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using

10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
3519.9.

20

EXAMPLE 188Preparation of Peptide having SEQ ID NO. 179

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly-
NH₂ [SEQ. ID. NO. 179]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3307.7.

EXAMPLE 189

10 Preparation of Peptide having SEQ ID NO. 180

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly-NH₂
[SEQ. ID. NO. 180]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
25 peptide. Electrospray Mass Spectrometry (M): calculated 3186.5.

EXAMPLE 190Preparation of Peptide having SEQ ID NO. 181

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 tPro Ser Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 181]

- The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Double couplings are required
at residues 37,36 and 31. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
15 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4121.1.

20

EXAMPLE 191Preparation of Peptide having SEQ ID NO. 182

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
25 Pro Ser Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 182].

- The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
30 Fmoc-protected amino acids (Applied Biosystems, Inc.),

195

cleaved from the resin, deprotected and purified in a similar way to Example 109. Double couplings are required at residues 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

- 5 Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4173.2.

10

EXAMPLE 192

Preparation of Peptide having SEQ ID NO. 183

- His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
15 NMeala Ser Ser Gly Ala NMeala NMeala-NH₂ [SEQ. ID. NO. 183]

- The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
20 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a similar way to Example 109. Double couplings are required at residues 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical
25 RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.
Electrospray Mass Spectrometry (M): calculated 3796.1.

EXAMPLE 193Preparation of Peptide having SEQ ID NO. 184

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 hPro Ser Ser Gly Ala hPro-NH₂ [SEQ. ID. NO. 184]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. A double coupling is required
at residue 31. Used in analysis are Solvent A (0.1% TFA in
water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
15 (gradient 30% to 60% Solvent B in Solvent A over 30 minutes)
of the lyophilized peptide is then carried out to determine
the retention time of the product peptide. Electrospray
Mass Spectrometry (M): calculated 3871.1.

20

EXAMPLE 194Preparation of Peptide having SEQ ID NO. 185

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
25 Pro Ser Ser Gly Ala-NH₂ [SEQ. ID. NO. 185]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then

- 5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3750.2.

EXAMPLE 195

10 Preparation of Peptide having SEQ ID NO. 186

His Gly Asp Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-
NH₂ [SEQ. ID. NO. 186]

- 15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a
- 20 similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
- 25 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3408.8.

EXAMPLE 196Preparation of Peptide having SEQ ID NO. 187

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
5 Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 187]

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
10 Fmoc-protected amino acids (Applied Biosystems, Inc.),
cleaved from the resin, deprotected and purified in a
similar way to Example 109. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN).
Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent
15 A over 30 minutes) of the lyophilized peptide is then
carried out to determine the retention time of the product
peptide. Electrospray Mass Spectrometry (M): calculated
4120.6.

20

EXAMPLE 197Preparation of Peptide having SEQ ID NO. 188

Ala Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 188]

25

The above-identified amidated peptide is assembled on
4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
Fmoc-protected amino acids (Applied Biosystems, Inc.),
30 cleaved from the resin, deprotected and purified in a

similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4005.5.

EXAMPLE 198

Preparation of C-terminal carboxylic acid peptides
10 corresponding to the above C-terminal amide sequences
for Peptides having SEQ ID NOS. 100-166, 172-177,
179-180 and 185-188.

C-terminal carboxylic acid peptides corresponding to amidated having SEQ ID NOS. 100-166, 172-177, 179-180 and
15 185-188 are assembled on the so called Wang resin (p-alkoxybenzylalcohol resin (Bachem, 0.54 mmole/g)) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to that described in Example 109. Used in
20 analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry
25 provides an experimentally determined (M).

EXAMPLE 199Preparation of C-terminal carboxylic acid peptides
corresponding to the above C-terminal amide sequences
for Peptides having SEQ ID NOS. 167-171, 178 and 181-184.

- 5 C-terminal carboxylic acid peptides corresponding to
amidated SEQ ID NOS. 167-171, 178 and 181-184 are assembled
on the 2-chlorotritylchloride resin (200-400 mesh), 2% DVB
(Novabiochem, 0.4-1.0 mmole/g)) using Fmoc-protected amino
acids (Applied Biosystems, Inc.), cleaved from the resin,
10 deprotected and purified in a similar way to that described
in Example 109. Used in analysis are Solvent A (0.1% TFA in
water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC
(gradient 30% to 60% Solvent B in Solvent A over 30 minutes)
of the lyophilized peptide is then carried out to determine
15 the retention time of the product peptide. Electrospray
Mass Spectrometry provides an experimentally determined (M).

EXAMPLES A TO EReagents Used

- 20 GLP-1[7-36]NH₂ (GLP-1) was purchased from Bachem
(Torrance, CA). All other peptides were prepared using
synthesis methods such as those described therein. All
chemicals were of the highest commercial grade. The CAMP
SPA immunoassay was purchased from Amersham. The
25 radioligands were purchased from New England Nuclear
(Boston, MA). RINm5f cells (American Type Tissue
Collection, Rockville, MD) were grown in DME/F12 medium
containing 10% fetal bovine serum and 2mM L-glutamine.
Cells were grown at 37°C and 5% CO₂/95% humidified air and
30 medium was replaced every 2 to 3 days. Cells were grown to

confluence then harvested and homogenized using on a Polytron homogenizer. Cell homogenates were stored frozen at -70°C until used.

5 EXAMPLE A - GLP-1 RECEPTOR BINDING STUDIES

Receptor binding was assessed by measuring displacement of [¹²⁵I]GLP-1 or [¹²⁵I]exendin(9-39) from RINm5f membranes. Assay buffer contained 5 µg/ml bestatin, 1 µg/ml phosphoramidon, 1 mg/ml bovine serum albumin (fraction V), 10 mg/ml bacitracin, and 1 mM MgCl₂ in 20 mM HEPES, pH 7.4. To measure binding, 30 µg membrane protein (Bradford protein assay) was resuspended in 200 µl assay buffer and incubated with 60 pM [¹²⁵I]GLP-1 or [¹²⁵I]exendin(9-39) and unlabeled peptides for 120 minutes at 23 C in 96 well plates (Nagle 15 Nunc, Rochester, NY). Incubations were terminated by rapid filtration with cold phosphate buffered saline, pH 7.4, through polyethyleneimine-treated GF/B glass fiber filters (Wallac Inc., Gaithersburg, MD) using a Tomtec Mach II plate harvester (Wallac Inc., Gaithersburg, MD). Filters were 20 dried, combined with scintillant, and radioactivity determined in a Betaplate liquid scintillant counter (Wallac Inc.).

Peptide samples were run in the assay as duplicate points at 6 dilutions over a concentration range of 10⁻⁶M to 25 10⁻¹²M to generate response curves. The biological activity of a sample is expressed as an IC₅₀ value, calculated from the raw data using an iterative curve-fitting program using a 4-parameter logistic equation (Prizm, GraphPAD Software).

EXAMPLE B - CYCLASE ACTIVATION STUDY

Assay buffer contained 10 μ M GTP, 0.75 mM ATP, 2.5 mM $MgCl_2$, 0.5mM phosphocreatine, 12.5 U/ml creatine kinase, 0.4 mg/ml aprotinin, 1 μ M IBMX in 50 mM HEPES, pH 7.4.

- 5 Membranes and peptides were combined in 100 ml of assay buffer in 96 well filter-bottom plates (Millipore Corp., Bedford, MA). After 20 minutes incubation at 37°C, the assay was terminated by transfer of supernatant by filtration into a fresh 96 well plate using a Millipore vacuum manifold.
- 10 Supernatant cAMP contents were quantitated by SPA immunoassay. Peptide samples were run in the assay as triplicate points at 7 dilutions over a concentration range of $10^{-6}M$ to $10^{-12}M$ to generate response curves. The biological activity of a particular sample was expressed as
- 15 an EC_{50} value, calculated as described above.

EXAMPLE C - DETERMINATION OFBLOOD GLUCOSE LEVELS IN DB/DB MICE

- C57BLKS/J-m-db mice at least 3 months of age were
- 20 utilized for the study. The mice were obtained from The Jackson Laboratory and allowed to acclimate for at least one week before use. Mice were housed in groups of ten at $22^{\circ}C \pm 1^{\circ}C$ with a 12:12 light:dark cycle, with lights on at 6 a.m. All animals were deprived of food for 2 hours before taking
 - 25 baseline blood samples. Approximately 70 μ l of blood was drawn from each mouse via eye puncture, after a light anesthesia with metophane. After collecting baseline blood samples, to measure plasma glucose concentrations, all animals receive subcutaneous injections of either vehicle
 - 30 (10.9% NaCl), exendin-4 or test compound (1 μ g) in vehicle.

- Blood samples were drawn again, using the same procedure, after exactly one hour from the injections, and plasma glucose concentrations were measured. For each animal, the % change in plasma value, from baseline value, was
- 5 calculated.

EXAMPLE D - DOSE RESPONSE DETERMINATION OF
BLOOD GLUCOSE LEVELS IN DB/DB MICE

- C57BLKS/J-m-db/db mice, at least 3 months of age were
- 10 utilized for the study. The mice were obtained from The Jackson Laboratory and allowed to acclimate for at least one week before use. Mice were housed in groups of ten at 22°C ± 1°C with a 12:12 light:dark cycle, with lights on at 6 a.m. All animals were deprived of food for 2 hours before taking
- 15 baseline blood samples. Approximately 70 µl of blood was drawn from each mouse via eye puncture, after a light anesthesia with metophane. After collecting baseline blood samples, to measure plasma glucose concentrations, all animals receive subcutaneous injections of either vehicle,
- 20 exendin-4 or test compound in concentrations indicated. Blood samples were drawn again, using the same procedure, after exactly one hour from the injections, and plasma glucose concentrations were measured. For each animal, the % change in plasma value, from baseline value, was
- 25 calculated and a dose dependent relationship was evaluated using Graphpad Prism™ software.

EXAMPLE E - GASTRIC EMPTYING

- The following study was and may be carried out to
- 30 examine the effects of exendin-4 and/or an exendin agonist

compound on gastric emptying in rats. This experiment followed a modification of the method of Scarpignato, et al., Arch. Int. Pharmacodyn. Ther. 246:286-94, 1980. Male Harlan Sprague Dawley (HSD) rats were used. All animals were housed at 22.7 ± 0.8 C in a 12:12 hour light:dark cycle (experiments being performed during the light cycle) and were fed and watered *ad libitum* (Diet LM-485, Teklad, Madison, WI). Exendin-4 was synthesized according to standard peptide synthesis methods. The preparation of exendin-4 is described in Example 14. The determination of gastric emptying by the method described below was performed after a fast of ~20 hours to ensure that the stomach contained no chyme that would interfere with spectrophotometric absorbance measurements.

Conscious rats received by gavage, 1.5ml of an acaloric gel containing 1.5% methyl cellulose (M-0262, Sigma Chemical Co, St Louis, MO) and 0.05% phenol red indicator. Twenty minutes after gavage, rats were anesthetized using 5% halothane, the stomach exposed and clamped at the pyloric and lower esophageal sphincters using artery forceps, removed and opened into an alkaline solution which was made up to a fixed volume. Stomach content was derived from the intensity of the phenol red in the alkaline solution, measured by absorbance at a wavelength of 560 nm. In separate experiments on 7 rats, the stomach and small intestine were both excised and opened into an alkaline solution. The quantity of phenol red that could be recovered from the upper gastrointestinal tract within 20 minutes of gavage was $89 \pm 4\%$; dye which appeared to bind irrecoverably to the gut luminal surface may have accounted

for the balance. To account for a maximal dye recovery of less than 100%, percent of stomach contents remaining after 20 min were expressed as a fraction of the gastric contents recovered from control rats sacrificed immediately after
5 gavage in the same experiment. Percent gastric contents remaining = (absorbance at 20 min)/(absorbance at 0 min) x 100.

Various modifications of the invention in addition to those shown and described herein will become apparent to
10 those skilled in the art from the foregoing description and fall within the scope of the following claims.